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Mating strategies and resulting patterns in mate guarding crustaceans: an empirical and theoretical approach

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General introduction

1. Mechanisms of sexual selection

Sexual selection occurs whenever individuals are in competition for access to reproduction (Andersson & Iwasa 1996). When competition happens over mates, individual's access to mate is constrained by the mating success of competitors or by competitors themselves through contests over mates (Andersson 1994). On the other hand, competition can also happen over fertilization. If reproductive partners mate with multiple individuals, a strong competition occurs for the fertilisation of their gametes (Parker 1970). Several mechanisms enhance sexual selection through competition for reproduction under two main conditions: (i) when mates are scarce in space or time, individuals should strongly compete for their prior access and (ii) when potential partners reject some mating attempts, individuals should compete to avoid rejection (Andersson 1994).

Sexual selection essentially occurs in males (Bateman 1948). Males have small numerous gametes which are rapid and cheap to produce. Comparatively, females have a few large gametes which require a long time and greater energy to produce. At any given time, male gametes are more abundant than female gametes leading to competition and sexual selection in males (Bateman 1948, Trivers 1972). Traits conferring males with a fertilization advantage such as larger ejaculates invested in each mating (Parker 1970), mate monopolization after mating (Alcock 1994) or mating plugs which prevent females from mating with competitors (e.g. Baer et al. 2001) will be sexually selected.

If females are dispersed in space and/or time, a lot more males are ready to mate than females, leading to male-biased operational sex ratio (OSR, i.e. the ratio of the number of sexually available males over the number of sexually available females, Emlen & Oring 1977) and strong competition among males for access to females. Sexual selection will then favour traits that increase male's chance to gain access to mate in contests with competitors and/or

traits that increase male's ability to find a mate before competitors. For instance, traits such as weaponry and large bodies, providing males with a competitive advantage in agonistic interactions with other males, are selected. On the other hand, sexual selection also leads to the evolution of greater sensory abilities or organs for efficient locomotion to locate mates (reviewed in Murphy 1998). Similarly, if females are only receptive for copulation for short periods of time, it pays males to present traits ensuring prior access at the time of their receptivity. For example, in species with definite breeding season as it is the case in several birds, males arrive earlier than females at the breeding site in response to competition for territories (reviewed in Morbey & Ydenberg 2001). This adaptation called protandry also exists in insects where males maximise their mating opportunities by maturing earlier than females (Iwasa et al. 1983).

Sexual selection also results from mating biases expressed by females that generate difference in mating success among males (Kokko et al. 2003). Females discriminate among potential males before mating, choosing partners of preferred particular phenotypes. This mate choice creates non-random mating in males and leads to strong male-male competition for access to reproduction. The resulting sexual selection favours the evolution of male traits that increase their probability to be chosen by females. Males will present ornaments and/or elaborate displays whose evolution is partly driven by female mating preferences (Pomiankowski et al. 1991). Alternatively, mating biases can arise if females do not actively reject males of given phenotypes but resist mating *per se* (Kokko et al. 2003). Mating is sometimes costly for females who evolve resistance behaviour in an attempt to avoid these costs (Parker 1979). In that case, competition increases in males, hence leading to sexual selection (Gavrilets et al. 2001). In response, males evolve persistence in their mating attempts in order to overcome females' resistance, sometimes leading to coercive mating.

It is worth pointing out that mate availability and mate choice are not two exclusive mechanisms which drive competition and sexual selection. If, due to low opportunity for reproduction, males compete for access to mates, females will end up having potentially access to several mates at a given time. In that case, it pays females to become choosy (Kokko & Monhagan 2001). Also, competition for fertilisation creates less paternity insurance among males because female's eggs are susceptible to be fertilised by competitors. Males are therefore less prone to provide parental care (Queller 1997). In species where parental care is necessary for offspring survival, it is females that will thus care most. In that case, females

become less available for reproduction at any time, hence increasing competition and opportunity for sexual selection in males (Kokko & Jennions 2003). Females can afford to be choosy because a lot of males are available to copulate with them at any given time which further increases competition among males to be chosen.

2. The evolution of female and male mate choice

Time and energy devoted to one reproductive event comes at a cost to future reproduction. Lifetime reproductive success of individuals investing heavily in each mating therefore depends on the success of a few reproductions. Potential mates sometimes vary greatly in the benefits they provide for reproduction. In that case, it pays individuals to choose mating partners associated with great fitness payoffs (Parker 1983, Kokko & Monhagan 2001). The evolution of preferences for particular traits of mates is driven by the fitness benefits associated with these traits. Preferences can target partners' traits associated with direct benefit for individuals' life-time fitness. For example, females evolve preferences towards fertile males or towards males which provide them with food, breeding site, paternal care, or protection against harassment (Iwasa & Pomiankowski 1999). Preferences can also evolve towards traits associated with indirect benefits. Females mating with fitter males will have offspring that inherit the male's good genes associated with higher survival and/or reproduction (Kirkpatrick & Ryan 1991, Kokko et al. 2003). Mate choice is also likely to evolve in the sex that suffers less competition for access to mates because the opportunity to find receptive mates is high and the cost of rejecting a partner is low. Mate choice has mainly been thought to evolve in females because they compete less than males for access to reproduction and because they are likely to spend substantial time and energy in each mating, by providing parental care for instance (Trivers 1972).

However, this view has been challenged in recent years with an increasing number of studies reporting examples of choosy males (see Bonduriansky 2001 for a review). Although costly parental investment is thought to be the main condition for the evolution of female mate choice, it cannot generally account for the situations of male mate choice as males usually provide less parental investment than females (Trivers 1972). Other conditions favor male mate choice though. First, if females vary widely in quality, it should become beneficial

to discriminate them and seek for high quality mates. This is of particular interest for the study of male mate choice considering that females often greatly vary in quality, sometimes even to a larger extent than males (Edward & Chapman 2011). Second, if the cost of searching for potential mates and assessing their quality is relatively low, individuals should become choosy (Kokko & Monaghan 2001, Bonduriansky 2001, Kokko & Johnstone 2002). Population density as well as mate sampling and assessment strategies used by males during decision making are thus expected to affect male choosiness. Third, if mating is costly for males in terms of future reproductive success, they should seek high quality females to mate with. For instance, long lasting displays are generally associated with greater mortality due to energy loss or increased predation risk. Similarly, males who invest heavily in sperm competition may require more time to reform their sperm stock to mate again. The evolution of costly giant sperm in *Drosophila* has been shown to reduce sexual selection imposed on males (Bjork & Pitnick 2006). When male adaptations to sexual selection impede the prospect of future reproduction by lowering their mating rate or increasing mortality, males should become choosy (Kokko et al. 2012).

3. Sexual selection and mating patterns

Mating biases such as preferences and resistance are not easy to detect in natural populations. Several researchers use observations of mating patterns to get information about the underlying mechanism that leads to it. This approach has flaws because several mechanisms may lead to the same mating pattern. This is the concept of equifinality (Burley 1983). Burley (1983) also presented the concept of multifinality according to which multiple patterns can result from a given mechanism. Burley presented these two concepts for the study of a well-described mating pattern: assortative mating. Assortative mating occurs when individuals of similar phenotypes mate more often than expected at random. In an attempt to explain the cause of such pattern, researchers often simply assume that individuals prefer to mate with alike, a preference called homotypic. Burley claims that this shortcut is misleading as assortative mating can also result from a directional preference; i.e. when individuals in a population share a preference for partners of a specific phenotype. Let us consider a population where high quality individuals are of phenotype A while low quality individuals are of phenotype B. Every individual, either males or females, prefers to mate with partners of

phenotype A. High quality males and females will accept each other as mates but will reject B individuals. B individuals will therefore have no choice but to mate with each other hence presumably leading to assortative mating. In her paper, Burley calls violations of equifinality "inferential fallacies" and strongly warns researchers to avoid them.

However, Burley implicitly considered that as soon as individual preferences are known and a particular mating pattern is observed at the population level, one can safely conclude that these preferences are responsible for the given pattern. However, this inference may not always stand. Even if we know for sure phenotypes targeted by a preference, it does not necessarily means that choosy individuals will eventually mate with preferred mates. This is because pairing processes are subject to constraints (Wagner 1998, Cotton et al. 2006). For instance, when every individual prefers to mate with partners of a specific phenotype (i.e. directional preference), competition for access to these partners is strong. As a consequence, choosy individuals may not all satisfy their preference.

Precopulatory mate guarding is a male coercive behaviour in response to strong male-male competition for access to rare receptive females. It represents a fascinating behaviour to study male mate choice, pairing process and their influence on pairing patterns. It is the focus of the present manuscript and will be now presented more extensively.

Chapter 1

Precopulatory mate guarding in crustaceans: love me tender, love me long.

1. Mate guarding

In several human societies, it is the responsibility of parents or elder members of the family to choose children's spouses for life (Apostolou 2007). These arranged marriages occur particularly in the Middle East, India, South East Asia or Africa. Children are sometimes married very young to people of the same age or older. Some are married before the age of 10 or a little bit later, just after they reached sexual maturity. The causes of these early marriages are multiple and mostly related to economic considerations. Young girls are seen as an economic burden and are married to older men that can provide for them instead of the family. In other situations, early marriage are arranged to ensure protection of young girls by male guardian, against undesired sexual relationships and illegitimate pregnancies. Although causes for such pairing processes are likely to be cultural, it resembles the well-known evolved pairing behaviour in animals that is mate guarding.

Mate guarding is described as a mate monopolisation strategy usually performed by males. It involves one or several males guarding one or several females and can occur either after (i.e. postcopulatory mate guarding) or before mating (i.e. precopulatory mate guarding). During mate guarding, males either stay at close proximity to their female and defend her from other males or initiate physical contact with her, usually by holding on to her. It has evolved under different constraints in many taxa. For instance, postcopulatory mate guarding has mainly evolved as a response to male-male competition for fertilization of eggs (Alcock 1994). In many species, females mate multiply which leads to sperm competition between

different male gametes inside female genital tract (Birkhead & Møller 1998). In response to such competition, males sometime guard females after copulation, therefore preventing them to re-mate with other males and securing their paternity over the brood (Alcock 1994). Such forms of mate guarding are particularly well described in mammals (Brotherton & Komers 2003, Komers et al. 1994, Huck et al. 2004, Schubert et al. 2009) and in birds where females often seek extra pair copulations (Møller & Birkhead 1991, Komdeur 2001). One well-known example is given by harems in elephant seals, where larger dominant males guard and mate with several females to prevent sneaky copulations from lower ranked males (Le Boeuf 1974). Postcopulatory mate guarding is also common in invertebrates, especially in insects where it functions to allow multiple copulations by males to ensure paternity of female eggs (Arnqvist 1989, Watson et al. 1998).

Under rather different constraints, mate guarding can also happen before copulation. This later case of mate guarding is the main subject of this work and will now be presented more extensively.

1.1 Where does precopulatory mate guarding exist?

Depending on the species where it is described, precopulatory mate guarding takes different names and relates to different mating strategies. Herein, I will thus briefly review precopulatory association between mating partners within three groups of animals where it has been observed: amphibians, insects and arachnids. Although their behaviour is of great interest for the theory tackled in the present work, these taxa are not the focus of our experiments, which we conducted in an amphipod crustacean. I will provide a more detailed review about the reproductive biology of crustaceans and about their precopulatory mate guarding behaviour after the following section.

1.1.1 Precopulatory mate guarding in anurans and Urodela:

In toads and frogs, mate guarding has often been reported during breeding seasons (Davies & Halliday 1979, McLister 2003). In these species, guarding is called amplexus which comes from "amplecti" that literally means "an embrace" in Latin. Males usually grasp females with their front legs to secure reproduction under strong male-male competition. In the common toad *Bufo bufo* for instance, males arrive before females at the breeding site and spawning usually occurs several days after arrival (Höglund 1989). As a consequence, when

females start to arrive, sex ratio is biased towards males and a lot of interferences between single and amplexed males occur (Davies & Halliday 1979). This sometime leads to the displacement of an amplexed male by a competitor, a behaviour called "takeover", or to several males being amplexed with the same female (Davies & Halliday 1979).

Newts and salamanders also present amplexus although they differ from those of anurans in their form. Males have been described to hold females with their legs and/or their tails by facing them ventrally or by riding them on her back (Halliday 1990). Amplexus is viewed as a courtship strategy in these species and can have a role in spermatophore transfer, or functions to increase female receptivity to mating (Halliday 1990). In the red-spotted newt (*Notophthalmus viridescens viridescens*), amplexus has also been reported to ensure female's defence against interference from competitor males during fertilization (Gabor et al. 2000).

1.1.2 Precopulatory mate guarding in insects and arachnids:

Although insects mainly display postcopulatory mate guarding, in a few species males have been showed to guard females before copulation. They sometimes guard females that are not sexually mature yet at the late stage of their last moulting cycle. For instance, in the Zeus bugs *Phoreticovelia disparata*, males ride fourth instar juvenile females on their back for several hours before they reach sexual maturity (Arnqvist et al. 2007, Jones et al. 2010). Analogous precopulatory behaviour is found in arachnids where males remain in webs of immature virgin females for several days and defend them against competitors to ensure reproduction when they reach maturity (Bel-Venner & Venner 2006).

In certain insect species, precopulatory mate guarding of adult and sexually mature females also occurs. In the cricket *Gryllus bimaculatus*, males transfer spermatophores during copulation, hence facing sperm depletion and a short reproductive time out right after mating (Parker & Vahed 2010). When males encounter by chance a single female while still forming a new spermatophore, they can guard her until they are ready to mate again, which takes about an hour (Parker & Vahed 2010). In a little coleopteran, the green chafer *Anomala albopilosa sakishimana*, males also guard females before copulation because those only mate during a short period of about 2 hours within the day. This behaviour provides the male with prior access to copulation at the time of female receptivity in face of strong male-male competition over mating (Arakaki et al. 2004).

Except for this latter case, precopulatory mate guarding in insects and arachnids takes place before females' sexual maturity. Females are thus guarded once in their life. This is

fairly different from the behaviour of crustaceans where males guard adult females when they are in a phase of non-receptivity to mating. Although insects and arachnids provide good examples of precopulatory mate guarding, the literature dealing with such behaviour is far more important in crustaceans. Mate guarding crustaceans are the subject of the next section.

1.2 Precopulatory mate guarding in moulting crustaceans

"This same naturalist [Charles Spence Bate[1]] *separated a male sand-skipper (…),* Gammarus marinus, *from its female, both of whom were imprisoned in the same vessel with many individuals of the same species. The female, when thus divorced, soon joined the others. After a time the male was put again into the same vessel; and he then, after swimming about for a time, dashed into the crowd, and without any fighting at once took away his wife. This fact shews that in the Amphipoda, an order low in the scale, the males and females recognise each other, and are mutually attached."*

C. Darwin 1874, pp 270

Precopulatory mate guarding is often simply referred to as "mate guarding" or "mate monopolization" in many taxa such as insects and arachnids, maybe because it does not always involve a male physically grasping a female. In amphibians, it is mainly called "amplexus", a word that had pass to the crustacean literature because many useful concepts it uses come from mate guarding in amphibians. Other terms such as "precopula" or the less employed "precopular" (e.g. Hume et al. 2005) have also been extensively used in crustaceans. "Precopula" is the term I will mostly employ due to its strong connections to the empirical and theoretical literature about precopulatory mate guarding in crustaceans.

In his book, Ridley (1983) applied the comparative method to the study of precopulatory mate guarding. For that purpose, he made a nearly exhaustive review of the literature about mating associations in arthropods and anurans. Among papers dealing with crustaceans, he found 101 species described as presenting a precopulatory mate guarding phase and 78 species that did not present one. Most belonged to (i) Branchiopoda which are known to comprise brine shrimps, water fleas, tadpole shrimps and clam shrimps, (ii)

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[1] Charles Spence Bate was a famous British naturalist (1819-1889), elected a fellow of the Royal Society in 1861 for his knowledge about the biology of crustaceans. He maintained a correspondence with Charles Darwin who often cited him in his books.

Copepoda which are mainly parasitic or planktonic small crustaceans and (iii) Malacostraca, the taxon comprising most crustacean species including crabs, lobster, shrimps and Peracaridae which are the model taxon for the present work. Since Ridley (1983), the research on precopulatory mate guarding crustaceans has been, on a large majority focused on three groups: hermit crabs (e.g. Goshima et al. 1998, Wada et al. 1999, Wada et al. 2011), isopods (e.g. Shuster 1981, Verrel 1985, Jormalainen & Merilaita 1993, Sparkes et al. 1996, Jormalainen & Shuster 1999) and most notably amphipods (e.g. Greenwood & Adams 1984, Ward 1986, Elwood et al. 1987, Iribarne et al. 1995, Dunn 1998, Bollache & Cézilly 2004a, Cothran 2004).

In our work, we focused our investigations on amphipods crustaceans. Among Peracaridae, the super-order which comprises amphipods and closely related isopods, Ridley found 56 species presenting a precopulatory mate guarding phase whereas only 6 did not. This shows the high prevalence of precopulatory mate guarding in these species and explains why they have been the subject of most of the literature about it (Jormalainen 1998). The next section will mainly present the biology of amphipods crustaceans and more precisely of the species *Gammarus pulex* (in the manuscript, we will refer to it as gammarids as a vernacular name for the taxon). However, it can for a good part be generalised to many taxa of other well-studied moulting crustaceans such as isopods, copepods and decapods (Ridley 1983).

1.2.1 General considerations

In order to understand the evolution of precopulatory mate guarding in crustaceans, one must understand their reproductive biology. Freshwater gammarids live in streams, rivers, ponds and lakes of Eurasia and America. The genus *Gammarus* contains over 200 described species. However, a lot more species are likely to be discovered in the future thanks to new molecular techniques that help to unravel the important cryptic diversity existing in this taxon (e.g. Witt et al. 2006, cf chapter 3). Gammarids are extensively studied in several fields of biological sciences. It is particularly well-studied in ecotoxicology research because it represents a good indicator of water quality. In ecology, it is a model taxon for studying biological invasions (Bollache et al. 2008, Piscart et al. 2009). It is also extensively studied in evolutionary biology. For instance, many species are intermediate hosts for manipulative parasites that alter gammarids' behaviour in order to increase their predation by the parasite's definitive host (Lagrue et al. 2007). However, my interests are more directed towards the wide literature dedicated to gammarid sexual behaviours. In the second edition of his book, *The*

descent of man, and selection in relation to sex (1874), Charles Darwin presented one of the first records of interrogations about the evolution of amphipods sexual behaviour. In a Brazilian amphipod *Orchestia darwinii*, he was wondering how the evolution has led to two distinct morphs of males, both different from the female's morph. Darwin (1974, pp. 265) also recognized that: "*Unfortunately the habits of crustaceans are very imperfectly known, and we cannot explain the uses of many structures peculiar to one sex*". Since Darwin, we fortunately know more about crustacean biology and the evolution of its sexual behaviour.

Reproductive biology

Gammarids grow continuously all along their lives after each moulting episode. Between two moults, during a period called the intermoult[2], individuals renew their cuticle (Cornet et al. 2012). Moulting is under the control of a steroid hormone called ecdysone (or crustecdysone) (Borowsky 1980). Ecdysone's titer increases during female moulting cycle. It reaches a pic at the end of intermoult before it drastically decreases and moulting occurs (Skinner 1985).

Reproduction is tightly linked to female moulting cycle. Gammarids are iteroparous and broods are produced at almost every moulting cycle. During their moulting cycle, females produce eggs in their ovaries in the dorsal part of their pereon (figure 1). Between two moults, their cuticle is too hard to allow eggs to pass through their oviduct (Sutcliffe 1992). Right after moult, the oviduct wall is soft enough to allow migration for fertilisation of newly produced eggs in their brood pouch situated on the ventral part of their body, in-between their coxal plates (figure 1). Oviposition (i.e. egg migration in the brood pouch) thus only occurs within the short period of time between moult and the hardening of the new cuticle. Females are therefore only receptive for copulation right after their moult and for only a relatively short amount of time. Depending on the species, this period of sexual receptivity varies from a few hours in *G. pulex* to more than 15 days in *Niphargus sp* which lives in caves (Ridley 1983). Punctually, females perform a moulting cycle solely intended to growth during which they do not produce eggs and thus do not reproduce (Souty-Grosset et al. 1998, Sparkes et al. 2000). However, the relative frequency of such growth moults compared to reproductive moults is not well described. Moulting cycle length is positively correlated to body size in both males and females as larger bodies require more time in order to reform a new cuticle

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[2] Intermoult also refers to a specific period of the female moulting cycle that takes place between the previous moulting event and the start of the premoult stage (Cornet et al. 2012). In this manuscript, we will use the term intermoult only to denote the period of time between two successive moult.

during moulting cycle. Also, females are not synchronous in their moulting time within a population. At any given time, only a small proportion of females are actually receptive for copulation (this proportion roughly corresponds to the opposite of the mean length of female moulting cycle 1/E(T), cf chapter 2).
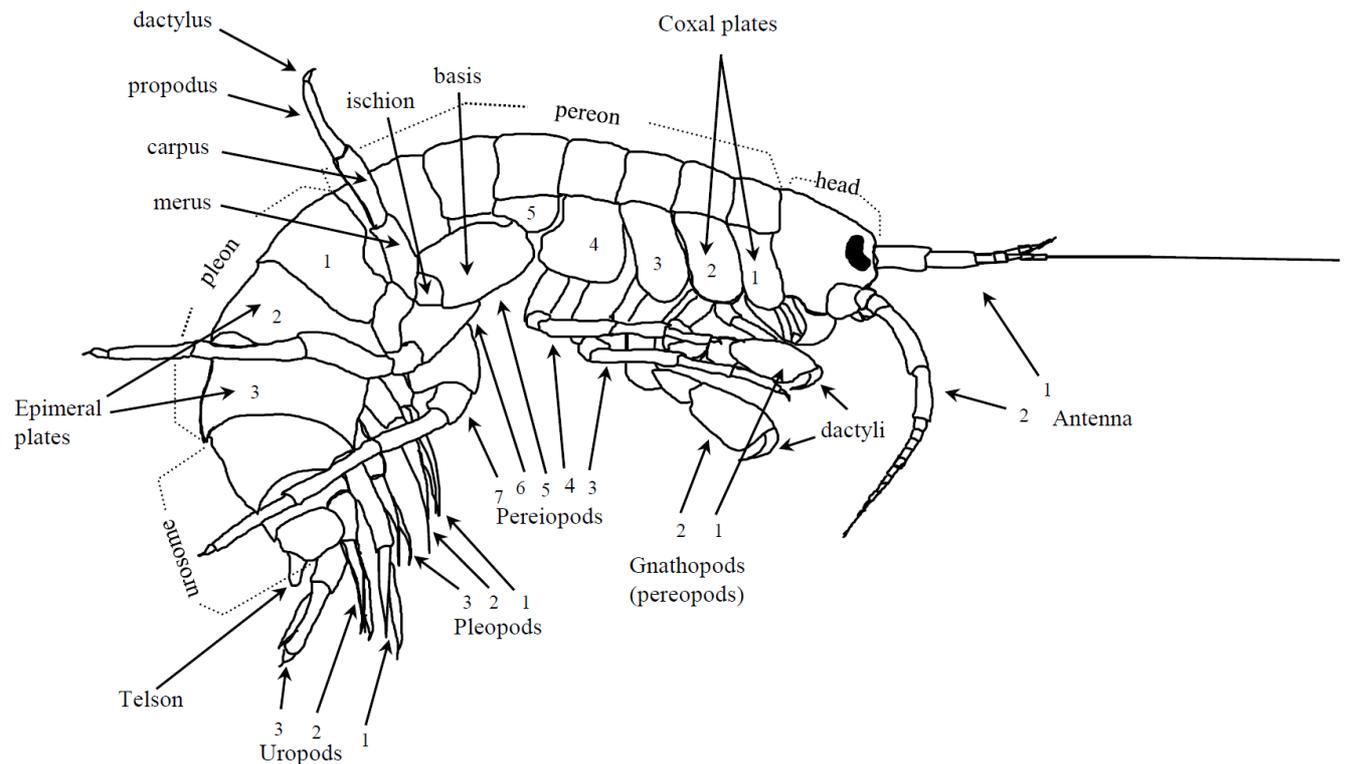
Figure 1: Gammarid morphology. Adapted from Roux (1971)

Fecundity varies with female body size. Sutcliffe (1993) provided a clear and detailed review of the literature on female fecundity in gammarids. Large females produce more eggs compared to smaller females (Hynes 1955, Birkhead & Clarkson 1980). The statistical link between body size and egg number have been suggested to be either linear or following a power or an exponential function (Sutcliffe 1993). Egg volume is also assumed to increase with female body size, although there seem to be a trade-off between the number of eggs produced by a female of particular size and their volume. Unfortunately, Sutcliffe (1993)

hardly presented any information about the variance of egg number and volume within each female size class.

Between two successive reproductions, females also carry young gammarids in their brood pouch from the copulation that took place at the previous moult. In the brood pouch, fertilised eggs develop into young fully developed gammarids during the course of the female moulting cycle and are released into the environment just preceding the next moult. Females perform maternal care to their offspring during their development, oxygenating them regularly by creating a current flow into their brood pouch and removing non-viable embryos (Dick et al. 1998, 2002).

Precopula

Precopulatory mate guarding takes place during female's intermoult. Before entering in precopula, males have to encounter a female. It has been suggested that waterborne pheromones function as attractant for males towards conspecific females (Dahl et al. 1970, Hammoud et al. 1975, Borowsky 1991). In addition, authors have suggested that contact pheromones may also exist, presumably allowing males to assess female's intermoult period (Ducruet 1973, Borowsky 1991). One can only speculate about the nature of such pheromones. Borowsky (1991) showed that males were less attracted to females that were previously found unpaired compared to females previously paired with a male. She Hypothesised that unpaired females were too far from moult to be attractive, suggesting that a sexual pheromone associated with moulting cycle was involved in mate recognition. For instance, it is likely that ecdysone plays a role in sexual attraction (Hammoud et al. 1975).

Once a female found, males engage in a complex pairing sequence which has been described in *G. pulex* under laboratory conditions by Le Roux (1933), Birkhead & Clarkson (1980) and Dick & Elwood (1989) and occurs as follows. After encountering a female, the male attempts to grab her with his gnathopods (figure 1). If he succeeds, he then uses his first, most anterior pair of gnathopods to hold the female by putting his dactyli (i.e. little claws at the end of each gnathopod, figure 1) under the female's cuticle on the top of her first (using one gnathopod) and fifth (using the second gnathopod) segment of her pereon (figure 1). Females even possess on these locations specialized area that fits the shape of males' dactyli and work as a lock-on system (Platvoet et al. 2006). During this early phase of the pairing

sequence, the male holds the female perpendicular to his own body. In that position, he then starts to brush his antennas (figure 1) on female's body and flexes his abdomen towards the ventral part of the female. After this phase, the male can either reject the female or decide to engage in long lasting pairing by holding her parallel to his own body beneath his ventral surface until she moults and copulation occurs (figure 2).

The way males hold females in precopula does not vary between species of the genus *Gammarus* but it differs from precopula holds of other described taxa (Borowsky 1984). For instance, males of the closely related genus *Hyallela sp* hold females by putting their dactyli under the cuticle of the second coxal plate on both sides of the female (Borowsky 1984).

Males are usually larger than females (Ward 1986, 1988). Sexual dimorphism also occurs regarding the size of two pairs of gnathopods (figure 1). Gnathopods of the second, most posterior pair are particularly larger than those of females (Hume et al. 2005). The second pair of gnathopods (figure 2), does not play any role in grasping the female. However, it has an important function in copulation. Without this second pair, males are not able to initiate copulation with their female at the time of her moult (Hume et al. 2005).
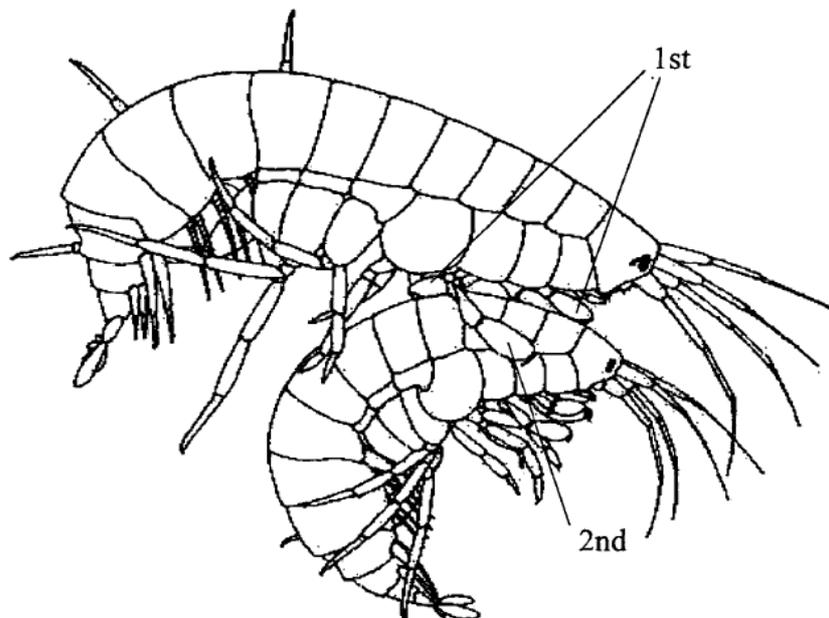
Figure 2: Typical parallel hold during precopulatory mate guarding in *Gammarus pulex*. The male (on top of the drawing) uses the first (1st) but not the second (2nd) pair of gnathopods to hold the female in precopula. From Hume et al. 2005

Female's sexual receptivity period

Soon after moult, females are sexually receptive and copulation can take place between the female and the male holding her in precopula. Fertilisation is semi-external and happens in the female's brood pouch. When initiating copulation, the male is returning to the perpendicular hold previously described. My own observation of the phenomenon made by filming copulations in *G. pulex* made me believe that it typically happens as follows. In order to inseminate sperm, the male vigorously flexes the posterior ventral part of his body towards the female's brood pouch. These flexes are performed in sequences of about 10 moves. Sequences are also repeated a few times (usually 4, or 5 times) being separated by short resting periods of a few seconds. Repeated sequences of body flexing constitute what I call an episode of copulation. They have been assumed to permit male's ejaculate to stick to the female's genital opening (Sutcliffe 1992). Between each episode, males get back to a parallel hold of their female. During female's sexual receptivity period, about 2 or 3 episodes of copulation are usually performed by the male (Heinze 1932, personal observations). However, I do not know whether sperm is transferred during each episodes of copulation. The overall copulatory behaviour hardly last more than 2 hours.

Shortly after the first episode of copulation, female initiate egg migration from their ovaries to the brood pouch where they presumably mix with the sperm. Egg's migration takes about 15 minutes to be completed. This means that late episodes of copulation actually occur while eggs are already in the brood pouch. Oviposition is plastic. If females are not in the presence of a male at the time of their moult, they can delay egg migration up to 15 hours after moult (Borowsky 1988, Borowsky 1991, personal observations). In that situation, larger females sometimes even begin a new moulting cycle without having laid eggs, whereas, smaller females always eventually lay their eggs into their brood pouch (personal observation). Females do not store sperm in gammarids (Borowsky 1991) so that they have to copulate with a male after each reproductive moult. If some eggs have not been fertilized after reproduction, females can resorb them, maybe as an energy recycling strategy (Ridley 1983). After reproduction, males usually leave their female. However, when a male competitor is present at proximity of the couple, males sometimes perform a short postcopulatory mate guarding (personal observation). Although males usually evolve this behaviour to secure paternity, there is no second male sperm precedence in gammarids and there is no description of any sperm ejection by competitor males that could hamper their fertilization success. Even more surprising is the study by Birkhead & Pringle (1986) that showed that the first male to

copulate with the female fertilises about 90% of the eggs, the rest being potentially fertilised by another male. We do not know the function of postcopulatory mate guarding but it might support the hypothesis of the presence of competition over fertilization in these species. On the other hand, numbers of copulatory flexes, sequences or episodes are not influenced by the presence of a competitor male close to the couple (personal observations).

1.2.2 The evolution of precopulatory mate guarding

The duration of precopula and the proportion of the female moulting cycle during which it occurs has been observed to widely vary between species, between populations of the same species or within populations (table 1) depending on environmental factors such as temperature and photoperiod (Sutcliffe 1992, Jormalainen 1998). However, is it worth pointing out that when measurements of precopula duration are made under laboratory conditions, some important factors influencing mate guarding may be missing (e.g. Kusano 1992). Under different conditions, individuals may adaptively change their mate guarding behaviour. So far, I have described the proximal causes for precopula. Knowing these mechanisms is of great importance when it comes to study the adaptive function of behaviours. In the next section, I will review the main hypotheses for the evolution of precopulatory mate guarding.

Precopulatory mate guarding duration, mate choice and male competitiveness

The evolution of precopulatory mate guarding has received much theoretical attention (e.g. Parker 1974, Wickler & Seibt 1981, Ridley 1983, Grafen & Ridley 1983, Yamamura 1987, Jormalainen 1998, Härdling et al. 2004). Research has mainly focused on understanding the evolutionary significance behind males' decision to guard females early in their reproductive cycle, hence leading to long lasting precopulatory mate guarding (Grafen & Ridley 1983).

Let us consider a population where females are only sexually receptive for a short amount of time during their reproductive cycle. Sexually receptive females are scarce in this population. Sexual selection would thus favour males that are able to detect the moment at which females are sexually receptive. Initially, mate guarding does not exist in the population so that males only pair up with females at the time of their reproduction. Because the

operational sex ratio (i.e. the ratio of males to females ready for copulation) is strongly male-biased, there should be a strong male-male scramble competition for access to receptive females. In that context, it should be strongly beneficial for a male to present an adaptation that provides him with prior access to receptive females. Let us assume a rare mutant male who guards encountered females one day before receptivity in a population where every male seeks receptive females. His behaviour should be sexually selected because it guarantees his access to reproduction after a short delay. His strategy will thus spread until every male in the population guards females one day before sexual receptivity. The scramble competition for these females thus becomes strong again. A mutant males starting guarding female even earlier in their reproductive cycle will be advantaged and his strategy will spread in the population. Although under long lasting mate guarding (i.e. earlier initiation of mate guarding in female's moulting cycle) the proportion of females considered to be suitable for paring increases, operational sex-ratio remains male biased. Females considered suitable for pairing are thus less frequent than are males available for pairing who are likely to always evolve longer lasting mate guarding. In that sense, the evolution of precopulatory mate guarding proceeds as a ratchet moving forward and reaching successive clanks; once males guard females for a given time before reproduction, it is almost impossible to start guarding females earlier in their moulting cycle. This process is assumed to continue towards longer durations of mate guarding until costs associated with precopula outweigh the competitive advantage it provides males with (Parker 1974, Wickler & Seibt 1981, Grafen & Ridley 1983, Jormalainen 1998). However, in theory, if it is not costly for males to guard females, precopulatory mate guarding can last for the whole female reproductive cycle and even lead to permanent monogamy (Wickler & Seibt 1981, Grafen & Ridley 1983, Brotherton & Komers 2003).

Precopulatory mate guarding has thus long been considered as a male competitive strategy in response to strong competition for access to receptive females (Jormalainen 1998). Actually, the fact that it is called "mate guarding" already assumes that males defend females against competitors (Ridley 1983). It has mainly been expected to occur when females are receptive to copulation for a short amount of time so that fertilization opportunities are really scarce for males (Parker 1974, Wickler & Seibt 1981, Ridley 1983, Grafen & Ridley 1983). This hypothesis has been later partly challenged by Yamamura (1987) who showed that guarding could actually evolve even when female period of receptivity is long lasting and as long as guarding is not too much costly for males. Precopulatory mate guarding also evolves even if females are synchronous in their receptivity period within the population (Yamamura & Jormalainen 1996).

However, it is worth pointing out that the evolution of such long lasting precopula under strong male-male scramble competition only applies to situations where males all seek females that meet a certain threshold of acceptance. In the simple verbal model I presented at the beginning of the section, every male in the population initially prefers females receptive for copulation. Male therefore show a mating preference for female proximity to reproduction. Mate guarding actually represents a male decision rule for male mate choice evolved under strong competition for preferred females. As a consequence, males paring with females closer to reproduction when searching costs are low (i.e. males have a high encounter rate of single females, Grafen & Ridley 1983) or when females vary in quality (Parker 1983). Practically, this view of the phenomenon does not reconsider the previously described theory according to which it is assumed to evolve. However, acknowledging that mate guarding evolves through male mate choice extends the field of possible investigations to understand variations in mate guarding durations. For example, we would expect that male mate choice based on multiple cues informing about female's quality to have an effect on mate guarding duration.

Also, decision rules for mate choice can depend on the condition of individuals exerting the choice (Riebel et al. 2010). For example, in mate guarding crustaceans, males have been described to display takeovers, hence usurping the female already taken in precopula by another male when she is close to reproductive moult (e.g. Ward 1983, Dick & Elwood 1990, Cothran 2008b). Larger males are assumed to be better able to perform these takeovers due to their competitive advantage in male-male agonistic interactions (Ward 1983). Therefore, authors have hypothesised that smaller males should start guarding females earlier in there moulting cycle compared to larger males who would rather tend to takeover females from smaller males when they are close to moulting (Grafen & Ridley 1983, Härdling et al. 2004). Apart from takeovers, other condition-dependent male guarding strategies may exist and influence mean guarding duration observed in populations (cf chapter 3).

Table 1: approximate precopula duration in certain species of amphipods. Modified from Jormalainen 1998

Species	Approximated precopula duration[a]	References
Gammarus duebeni	5 to 10 days in the lab (~28%) 6 to 28 days in the field (~48%)	Ward 1984, 1985, Sutcliffe 1992, Dick & Elwood 1996
Gammarus insensibilis	few hours	Thomas et al. 1995, 1996
Gammarus pulex	2 to 25 days in the lab (~40%) 7 to 30 days in the field (~54%)	Birkhead & Clarkson 1980, Ward 1983, 1984, 1986, Sutcliffe 1992, Hume et al. 2002, Plaistow et al. 2003
Gammarus lawrencianus	4 days (40%)	Hunte et al. 1985, Robinson & Doyle 1985
Gammarus zaddachi	4 days (24%)	Jormalainen & Merilaita 1995
Hyallela azteca	1 to 5 days (53%)	Welborn & Bartholf 2005, Cothran 2008a
Jesogammarus suvaensis	6 to 15 days (30%) in the lab 60% to 80% in the field	Kusano 1992
Eogammarus oclairi	up to 7 days (40%)	Iribarne et al. 1995
Microdeutopus gryllotalpa	1 day (~14%)	Borowsky 1980
Paracalliope fluviatilis	1 to 4 days	Sutherland et al. 2007

[a] depending on the study, precopula durations are given in days and/or in the percentage of female moulting cycle during which it takes place (between brackets)

Precopulatory mate guarding is a costly behaviour

Precopulatory mate guarding has not always been considered resulting solely from male behaviours in response to competition. In fact some authors have proposed that precopula may evolve to ensure female's fertilisation when they are receptive for a short time (Blegvad 1922, Le Roux 1933). Later, studies have also considered females behaviour in response to precopula attempts made by males and its effect on guarding duration (Jormalainen 1998). As explained above, males benefit from long guarding duration under

strong male-male scramble competition for access to females. However, this does not mean that they do not incur costs while performing such behaviour. They may lose some energy in precopula (Jormalainen et al. 2001, Plaistow et al. 2003) or be more subject to predation by fishes or insect larvae (Cothran 2004). In fact, guarding costs incurred by males have been shown to affect their decision rule (Grafen & Ridley 1983, Yamamura 1987, Yamamura & Jormalainen 1996). When guarding is costly, males should be choosier and start guarding females closer to their moult.

Females also suffer the risk of being predated while paired. In addition, they are sometimes cannibalised by their male partner (Dick 1995, Jormalainen 1998). To avoid these costs, they should prefer rather short precopulas initiated close to their moult (Jormalainen et al. 1994a). If the balance between costs and benefits associated to precopula is different between males and females, optimal guarding strategies should be different in the two sexes (Parker 1979). Optimal guarding duration is assumed to be greater for males than females thanks to benefits associated with guarding for males. An intersexual conflict is therefore likely to occur over the length of precopulatory mate guarding (Parker 1979, Jormalainen 1998). This is expected to lead female to evolve resistance towards males' attempts to initiate early precopula. In response, males may evolve persistence and coercion in order to overcome female reluctance to pair (Parker 1979, Arnqvist & Rowe 2005). According to theory, depending on factors such as the rate at which males encounter females, sex-ratio or synchrony in female moulting cycle, the resulting guarding duration may be either a perfect compromised strategy between males and females guarding optima or may be closer to the strategy of one or the other sex (Jormalainen et al. 1994, Yamamura & Jormalainen 1996). These predictions have been tested empirically (Jormalainen & Merilaita 1993, 1995, Watson et al. 1998, Jormalainen et al. 2000, Benvenuto & Weeks 2012). Studies revealed that, females of several species show resistance to early precopula that result in shorter mate guarding duration (e.g. Ridley & Thompson 1979, Shuster 1981, Thompson & Moule 1983, Jormalainen & Merilaita 1995). However, in other species, females barely resist precopula initiation (e.g. in *Gammarus zaddachi*, Jormalainen & Merilaita 1995) and theory is lacking to explain this. The strength of a sexual conflict over the optimal duration of precopulatory mate guarding in the two sexes is likely to have a major effect on the observed length of precopula. Different ecological conditions that affect the strength or the outcome of the conflict may explain the observed variation in precopulatory mate guarding duration between populations.

1.2.3 Precopulatory mate guarding and pairing patterns

Observing pairing patterns provides a first approach for the study of pairing processes. Evidences for non-random pairing such as phenotypic differences between paired and unpaired individuals as well as resemblance between mating partners with respect to certain traits, may provide useful but indirect information about the underlying mechanisms that might cause it. One extensively studied non-random pairing pattern is size-assortative pairing. It is defined as a positive statistical correlation between males and females body size in pairs (Ridley 1983, Crespi 1989, Cézilly 2004). Surprisingly, before the twentieth century, it was only studied in humans (Ridley 1983). Karl Pearson, famous for his product-moment correlation coefficient, was one of the first to measure positive homogamy for size in humans (Pearson 1899). He observed size assortment among 1000 husbands and wives from data provided to him by the Cambridge Anthropometrical Committee (r = 0.3, CI [0.24, 0.35], we measured this confidence interval using the fisher's'z method on Pearson's original results). From his observations, he tried to understand the mechanism causing such pattern. This is what he argued:

"Now there is little doubt that there is a certain amount of conscious assortative mating in this respect; a short man does not, as a rule, like a very tall wife."

K. Pearson 1899, pp 26

With this statement, he committed an inferential fallacy by affirming that size-assortative mating came from a male homotypic preference (i.e. males prefer to mate with females of similar phenotypes, Burley 1983). More importantly, his measures of homogamy were intended to study the consequences of such pattern on couple fertility. In that case, inferences about the potential link between male preference and fertility are particularly subject to caution. In a subsequent paragraph he also says:

"That in man, whether from conscious or unconscious sexual selection, there is far more homogamy than has hitherto been supposed, my family data cards amply demonstrate. If in man, then with great probability we can consider it to exist in other forms of life."

K. Pearson 1899, pp 32

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Pearson was right on this point, as homogamy is probably the most common mating pattern observed in nature. Since Pearson, it has been observed in other mammals (Pack et al. 2012) and many other taxa including birds (Delestrade 2001, Helfenstein et al. 2004), reptiles (Shine et al. 2001, 2003), fishes (McKaye 1986, Beeching & Hopp 1999, Baldauf et al. 2009, Taborsky et al. 2009), insects (Fairbairn 1988, Arnqvist et al. 1996, Harari et al. 1999, Jones et al. 2012), arachnids (Miyashita 1994, Hoefler 2007) and most notably crustaceans (for this last taxon, I will present the main literature about size-assortative pairing in chapter 3).

Long lasting physical associations between partners is often a prerequisite for observation of pairing patterns in the field. That is why size-assortative pairing is particularly well described in amphipods (e.g. Crozier & Snyder 1923, Birkhead & Clarkson 1980, Elwood et al. 1987, Bollache et al. 2000, Franceschi et al. 2010). Authors have long been interested in the mechanisms causing such pairing pattern and many hypotheses, which I will review in Chapter 3, have been put forward to explain it (Crespi 1989). Above all, mechanisms of mate choice have long been considered to explain size assortment. Male mate choice based on female body size is thought to play an important role in the occurrence of size-assortative pairing yet little is known about the evolution of such mate choice in mate guarding species. In fact, studies on precopulatory mate guarding have seldom linked males' evolved guarding criteria to patterns of size-assortative pairing observed in the field (but see Elwood & Dick 1990, Bollache & Cézilly 2004a).

It is worth pointing out that size-assortative pairing does not necessarily involve size-assortative mating as pairs may form only temporary and may split up before reproduction. In the present manuscript, we will use the term size-assortative mating or homogamy when individuals of a pair are known to associate until reproduction and size-assortative pairing otherwise.

Other noticeable patterns have been reported in gammarids. Males are generally larger than females in several species. This sexual size dimorphism is also observed among pairs with males being on average 30% larger than the female they are paired with (Adams & Greenwood 1983, Greenwood & Adams 1984, Ward 1986). It is also very common to observe size difference between paired and unpaired individuals. Males are usually larger in pairs compared to unpaired males (Birkhead & Clarkson 1980, Bollache & Cézilly 2004b). On the other hand, females in pair have sometime been reported to be smaller than unpaired females (Hatcher & Dunn 1997).

2. Aims of the presented work.

Precopulatory mate guarding in crustaceans provides a perfect matrix to study inferential fallacies. Although males evolve mate guarding in response to strong competition for access to receptive females, the time cost it involves may provide with prerequisite for the evolution of male mate choice. Mate choice is also assumed to be of great importance in creating specific mating patterns such as homogamy. Certain topics included in this manuscript have been extensively studied. However, we believe that some previous works may have made some shortcuts leading to over interpretations of the research outcome. We considered alternative but not exclusive explanations for well described patterns imbedded in the reproductive biology of mate guarding crustaceans. However, these new findings have some theoretical importance in other fields of animal behaviour and evolutionary biology. In the present work, we used theoretical and empirical approaches to study male mating strategies under the strong scramble competition imposed by time limited opportunities for fertilization.

In the second chapter, we will study the role of male mate choice strategies based on multiple female traits informing on their quality on the evolution of precopulatory mate guarding. Using a theoretical approach, we will first present a study of male decision rules in sequential mate sampling before entering in precopula. We will then consider male sampling strategies and criteria used for mate choice when it occurs while males are already paired with a female.

In the third chapter, after a review of the different mechanisms put forward to explain size-assortative pairing in natural populations, we will investigate the role of a male state dependent decision rule based on female proximity to moult on the occurrence and maintenance of such mating pattern. We will also present a study revealing some potential biases when surveying size-assortative pairing in the field in different cryptic populations of gammarids living in sympatry. More generally, our aim is to emphasize the potential for inferential fallacies when linking mating preferences to mating patterns.

In the fourth chapter, we will consider the effect of male guarding optimal duration on the occurrence of sexual conflict over guarding duration. Researches in reproductive strategies have suffered a strong gender bias in the study of males and females adaptations to sexual conflict (Karlsson Green & Madjidian 2011). In amphipods, authors have mainly

focused on costs for females associated with long lasting guarding durations. Here, we acknowledge several benefits that females could gain from being guarded.

I will present here the result of a collaborative work. Even though I took full responsibility for the researches undertook and wrote the associated articles, I received valuable theoretical and technical support from many people. It is for that reason that I will acknowledge the help I received at the beginning of each chapter by naming people that took part in each project. Although their contribution did not always lead to an article or a section presented in this manuscript, it markedly improved my understanding of biological phenomenon understudied.

Chapter 2

Mate choice and sampling rules: male choosiness before and during precopula

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1. Male mate choice before precopula

"The inherent plausibility of the hypothesis that mate choice is a common feature of the sexual behavior of animals should make us especially cautious and critical in our evaluation of attempts to demonstrate its occurrence in nature"

<div align="right">T.R. Halliday 1983, pp 3</div>

1.1 Introduction

Although studies of female mate choice prevail in the large majority of the sexual selection literature, in amphipods, researches are almost exclusively focused on male mate choice (but see Cothran 2008c). Amphipod mating system is described as a coercive polygynandry, which means that pairing decisions are mainly under the male's control and that both males and females mate multiply during their life with different partners (Shuster & Wade 2003). Mate choice has only been thought to provide males with direct benefits. Females' quality as sexual partners is based on two main female traits: their fecundity and their proximity to reproduction. Female fecundity is mainly determined by the number of her eggs and the amount of vitellus they contain (Sutcliffe 1992). It does not vary during female

moulting cycle although females that are not in the presence of a male at the time of their moult do not always lay eggs (Borowsky 1988). On the other hand, proximity to reproduction varies with time. A male pairing with a female while she is far from moult will see the quality of his partner increase with time (Hunte et al. 1985). Indirectly, fecundity positively correlates with females' intermoult duration because they both positively correlate with female body size. However, females of a given size can be at different times in their moulting cycle. Therefore, only a weak correlation exists between fecundity and female proximity to reproduction (see figure 1 in manuscript 2 for further explanations). A third female trait which is sometimes put forward in male mate choice studies is parasite load. In certain species, males do not pair with infected females probably because parasites negatively affect their fecundity (e.g. in *Gammarus pulex* females are castrated by an acanthocephalan parasite, Bollache et al. 2002). To my knowledge no studies reported any mate choice providing males with indirect fitness benefits.

Males assess potential partners' quality using different cues. Female's body size has often been considered as a reliable proxy of fecundity and males have been suggested to base their choice on this criterion rather than on female's fecundity itself (Dick & Elwood 1989). Very little is known about how males assess females' proximity to reproduction, but it is likely that hormones such as ecdysone or other chemical stimuli play a role in such assessment. Male mate choice has almost always been tested with the same protocol across species: unpaired males are usually placed in presence of two unpaired females differing in qualities. Individuals are then left to interact for a given time that varies between studies until the male eventually initiates precopula with one of the two females. Table 2 summarizes the results of different studies that tested male mate choice on one or both cues of female's quality in amphipods and isopods. Observations vary a lot between studies. Studies testing for the effect of female body size alone on male mate choice have either observed males choosing larger more fecund females or pairing at random with one of the two presented females. Studies testing the effect of both criteria of female's quality on mate choice have either reported males choosing on the basis of female's body size alone, female's proximity to reproduction alone or both. However, in one study, males seemed to be unselective before entering in precopula, pairing with the first contacted female (Goshima et al. 1998). Overall, the number of studies reporting mate choice of female's body size approximately equals the number of studies reporting mate choice on female's time left to moult. The lack of consistency between observations of male mate discrimination indicates potential differences

in the ability of males to assess female's relative qualities or environmental effects on benefits associated with male mate discrimination on one or the other cue.

It is worth pointing out that testing male preference on one female trait by allowing males to encounter simultaneously two unpaired females differing in relative quality may not necessarily predict pairings occurring in natural conditions (Wagner 1998). Mate choice results from mating preferences but also from other factors such as sampling rules used by individuals to have access to partners (Widemo & Sæther 1999). Under strong male-male competition for access to females, males may not be able to simultaneously encounter two unpaired females because most of the females are already paired with other males. In addition, mating preferences can be based on multiple criteria of mate's quality (Candolin 2003). If males base their choice on the two criteria of female's quality, it is possible that their preference threshold on female's body size influences their threshold on female's time left to reproduction. Only a few studies have considered such interaction between criteria in male mate choice in mate guarding crustaceans (e.g. Thompson & Manning 1981, Ward 1984a, Elwood et al. 1987). Authors argued that males should value potential partners by assessing the return in terms of eggs per day spent guarding they are associated with. When encountering two females simultaneously, males should prefer to pair with the female that provides them with the highest ratio of number of eggs over the time they will have to guard her before copulation (this ratio is sometimes referred to as female's utility, Elwood et al. 1987). However, little is known about the influence of this ratio on male mate choice when females are encountered sequentially.

Theoretical investigations of the evolution of precopulatory mate guarding have provided us with important insights on male mate choice according to female's time left to reproduction. Under an even sex-ratio, males are not predicted to be very choosy, guarding females early in their moulting cycle (Grafen & Ridley 1983, Yamamura 1987). In fact, when encountering females sequentially, it has even been suggested that males should pair with the first encountered female, regardless of her time left to moult (Grafen & Ridley 1983). However, these models did not consider the possibility for males to base their choice also on female's body size. Considering that males invest a lot of time in each mating by pairing with females early in their moulting cycle, they should make sure that the female they carry is fecund enough to compensate for the loss of mating opportunities. Males should therefore be choosier on female's body size when they are not very choosy on female's time left to moult.

We tested this hypothesis with a rate maximisation model considering that males were able to discriminate females on both criteria before entering in precopula. Individuals are classically thought to evolve mate choice when there is a high opportunity cost associated with each mating and/or when potential partner's quality varies substantially in the population (Parker 1983, Kokko & Monaghan 2001, Bateman & Fleming 2006). However, mate choice is usually described to hardly evolve when competition for mates is strong (Reading & Backwell 2007, Barry & Kokko 2010). Here we tested for these three parameters on the evolution of male mate choice on two criteria of female's quality in a context of precopulatory mate guarding. Contrary to previous models of the evolution of precopulatory mate guarding, we did not consider the effect of variation in males' competitive ability for access to females (e.g. Grafen & Ridley 1983, Härdling et al. 2004) or of female's resistance behaviour (e.g. Jormalainen et al. 1994a, Yamamura & Jormalainen 1996) on pairing outcome. In the next section, I will describe the model and its predictions regarding the evolution of precopulatory mate guarding and male choosiness under male-male competition.

Table 2: number of studies reporting male mate choice on female's body size or time left to moult (TLM) in different species of mate guarding crustacean

Tested criteria[a]	Choice on body size only	Choice on TLM only	Choice on both criteria	No choice	Tot
TLM alone[b]	-	1	-	0	1
Body size alone[c]	4	-	-	2	6
Both[d]	2	2	2	1	7

[a] females' body size and TLM were measured prior or after experiments. Authors assumed that males were able to assess these traits accurately before making a choice.

References: [b] Sparkes et al. 2000, [c] Manning 1975, Adams et al. 1989, Jormalainen et al. 1992, Iribarne et al. 1996, Sutherland et al. 2007, Dunn et al. 2008, [d] Birkhead & Clarkson 1980, Thompson & Manning 1981, Elwood et al. 1987, Dick & Elwood 1989, Jormalainen et al. 1994b, Goshima et al. 1998, Wada et al. 2011.

1.2 The model

Although our model originates from interrogations about the mating biology of mate guarding crustaceans, it can be applied to several mating systems where mating comes with an opportunity cost because there is some time between mate encounter and actual reproduction. Details about the R code of the following model can be found in appendix 2 (R development Core Team 2012). We considered a population where females do not reproduce continuously. Female reproductive cycle consisted of a period of reproductive "time-out" during which they could not reproduce, and a period of "time-in" during which copulation could occur. We considered the time-in to be very short, taking place at the end of each time-out period over several reproductive cycles during female's lifetime. At the end of a time-out, females reproduced and instantly began a new time-out period. We therefore considered that the length T of female reproductive cycle equalled the length of their time-out period. During their reproductive cycle, females could be at any stage t which takes discrete values between 1 and T. They changed stage at a rate g (by default $g = 1$). If they changed stage at $t = 1$, they reproduced and immediately began a new reproductive cycle at $t =$ T. Females had a probability of $P_c = 1 - e^{-g}$ to change from stage t to stage t-1. As a consequence, they reproduced and began a new reproductive cycle with a probability $(1/T) \times P_c$.

Within a population, females did not vary in the length T of their reproductive cycle (by default, T=40) but they were not synchronous in their receptivity period. As a consequence, within the female population, t followed a discrete uniform distribution of mean $(1 + T)/2$.

Females also varied in body size s within a population. As for t, we treated s as a discrete variable. By default, s took 40 values ranging from 0.025 to m, the maximum female size (by default, m=1). Female size followed a discrete quartic distribution $Q(s)$ described by this function:

$$Q(s) = s^3 (1-s)^3 \qquad (1)$$

s was also positively correlated to female's fecundity f according to this function:

$$f(s) = (as)^b + 1 \qquad (2)$$

where *b* controls the shape of the relationship between size and fecundity. The greater *b*, the more accelerating the function is and the larger females have to be in order to have fecundity greater than 1. This means that when *b* increased, only large females varied in fecundity because below a certain threshold of size, female's fecundity always equalled 1. When *b* tended towards 0 or $+\infty$, every female in the population tended to have the same fecundity. The other parameter of female's fecundity, *a*, controls the range of fecundity difference between females. When *a* increased, the difference in fecundity between the smallest and the largest female in the population increased. When *a* tended towards 0, every female in the population had a fecundity equal to 1. When both *b* and *a* were large, a few larger females had a fecundity greater than 1, and there was an important variation in fecundity among these females.

A population consisted of N_f females and of N_m males. Males encountered unpaired females at a rate λ. This means that males encountered each unpaired females λ times per time step of the model. A male having access to a particular female had to wait a time *t* before she became sexually receptive to copulate and he gained a fecundity f(s). While waiting, he guarded the female, rendering her unavailable to other males in the population. When the female became receptive, the couple instantly split up and males and females were immediately available for pairing with a new mate.

1.2.1 Male mate choice

Before pairing, males could choose females on the basis of their size *s* (as a proxy of their fecundity) and/or on the basis of their time left to reproduction *t*. Male mate choice strategy consisted of having a probability *P*(s,t) to pair up with a female of size s and of time left to reproduction *t*. *P*(s, t) was a matrix containing values ranging from 0 to 1 for each combination of values taken by *s* and *t*. For *P*(s, t) = 0, males rejected females. On the other hand, for *P*(s, t) > 0, males accepted females with a probability corresponding to the value of the matrix. Males did not choose females according to a particular threshold on *s* above which they paired with a female regardless of *t*. They did not have either a particular threshold of *t* below which they chose a female of any *s*. Instead, choosiness on one criterion could affect choosiness on the other criterion so that the acceptability of a female of particular size depended on her time left to reproduction.

Males mated over successive reproductive cycles. A cycle consisted of a male searching for a female, guarding this female until her period of sexual receptivity, copulating

with her and leaving her to begin searching again. The male beginning searching again corresponded to the renewal time of the cycle. This renewal time could occur either after the male left the female following copulation or after he rejected an unacceptable female. For each mating, a male thus gained a fecundity G and paid a time cost D. The time cost D corresponded to the time he spent searching for an acceptable female $1/\lambda$ and the time t he spent guarding her. Over several mating, the expected fecundity gain achieved by a male in a population where every male uses a strategy $P(s,t)$ was:

$$E(G) = \sum_{t=1}^{T} \sum_{s=0.025}^{m} f(s) \frac{n_{f-}(s,t)}{N_{f-}} P(s,t) \tag{3}$$

where $n_{f-}(s,t)$ is the number of unpaired females of a particular s and t and N_{f-} is the total number of unpaired females. On the other hand, the expected time cost this male paid was:

$$E(D) = \frac{1}{\lambda N_{f-}} + \sum_{t=1}^{T} \sum_{s=0.025}^{m} t \frac{n_{f-}(s,t)}{N_{f-}} P(s,t) \tag{4}$$

The left hand side of equation (4) represents the cost of searching for a suitable mate whereas the right hand side represents the time cost of guarding a mate until reproduction. Overall, the mean rate of fitness gain achieved by a male in a population of males using a strategy $P(s,t)$ was:

$$\gamma = \frac{E(G)}{E(D)} \tag{5}$$

In order to calculate γ, we had to calculate $n_{f-}(s,t)$ given that males used a strategy $P(s,t)$. The next section explains the procedure.

1.2.2 Feedback of male strategy on unpaired females' quality distribution

The change in the number of unpaired females at each t was controlled by: (i) the number of unpaired females at $t+1$ who changed stage without pairing (i.e who got 1 time step closer to reproduction, situation A, figure 3), (ii) the number of unpaired females at t who

changed stage without pairing (situation B, figure 3) and (iii) the number of unpaired females at t who paired up with a male without changing stage (situation C, figure 3). Females changed stage at a probability P_c and paired up at a probability $P_p = 1 - e^{-R(s,t)}$, where R(s,t) is the rate at which females paired up:

$$R(s,t) = N_{m-} \lambda P_i(s,t) \qquad (6)$$

where N_{m-} is the number of unpaired males in the population: $N_{m-} = N_m - N_f - N_{f-}$ (at the start of the model, $N_{m-} = N_m$).

Females that finished a reproductive cycle started a new one unpaired. At T, the number of unpaired females $n_{f-}(s, T)$ thus corresponded to the number of females $n_f(s,1)$ (paired or unpaired) of each size who changed stage plus those at T who did not pair up or change stage (situation D, figure 3). It was calculated as follows:

$$n_{f-}(s,T) = n_f(s,1)P_c + n_{f-}(s,T)(1-P_c)(1-P_p) \qquad (7)$$

The number of unpaired females of size s at any other stage corresponded to situation A plus D in Fig. 3 and followed this equation:

$$n_{f-}(s,t) = n_{f-}(s,t+1)P_c(1-P_p) + n_{f-}(s,t)(1-P_c)(1-P_p) \qquad (8)$$

In a nutshell, given a particular male mate choice strategy $P(s,t)$, unpaired females were always more likely to be at the beginning of their reproductive cycle thanks to the constant flow of unpaired females beginning a new reproductive cycle after recently being released from precopula.

1.2.3 Finding the optimal strategy P^*(s,t) of male mate choice

The optimal strategy P^*(s,t) of male mate choice maximised γ. Given the resident rate γ achieved by males with a strategy P(s,t), the best response by a mutant P'(s,t) maximises

$$E(G') - \gamma E(D') \qquad (9)$$

We used an iterative process in order to find the optimal strategy of mate choice. We started from a given value of the mean net rate of fecundity gain γ_1 calculated from equation (5) under a particular starting strategy $P_1(s,t)$. When every male in the population used the strategy $P_1(s,t)$, it affected the distribution of remaining unpaired females which we calculated accordingly with equations (7) and (8). Taking γ_1 as the resident mean rate of fitness gain in the population, we then calculated the mutant strategy $P_2(s,t)$ which maximised equation (9) considering the distribution of remaining unpaired females. After calculating γ_2 using $P_2(s,t)$ in equation (5), we iterated the process until $\gamma_i = \gamma_{i-1}$ meaning that $P_i(s,t)$ had stabilized. The male mate choice strategy $P^*(s,t)$ that maximised equation (9) with $\gamma = \gamma^*$ was the evolutionary stable optimal strategy.
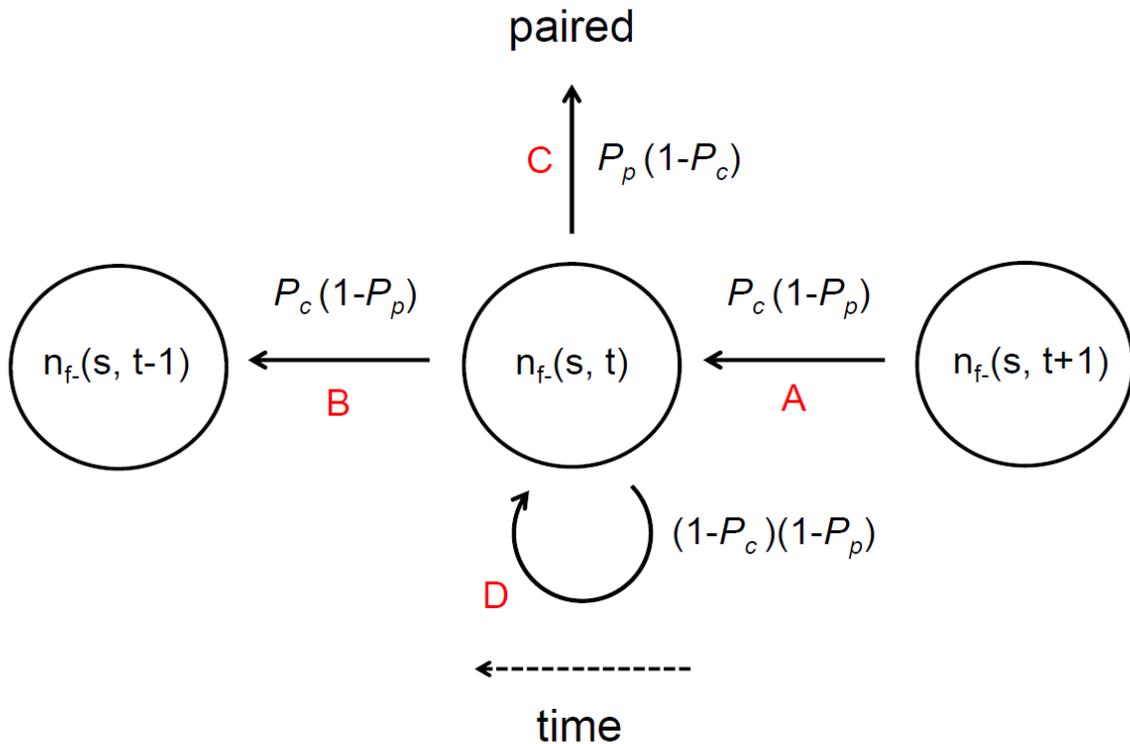
Figure 3: Path diagram showing the different ways in which the number of unpaired females $n_{f-}(s, t)$ of given size s at stage t of their reproductive cycle can change. Situation A represents the inflow of unpaired females of same size but from stage $t+1$ who change stage without pairing up with a probability $P_c(1-P_p)$. Situation B represents the outflow of unpaired females at t who change stage without pairing up at a probability $P_c(1-P_p)$. Situation C represents the outflow of unpaired females at t who pair up without changing stage at a probability $P_p(1-P_c)$. Situation D represents unpaired females at t who do not pair up and do not change stage at a probability $(1-P_c)(1-P_p)$.

1.3 Results

1.3.1 Optimal mate choice strategy

At γ^*, males had reached an optimal strategy P^*(s,t) of mate choice. Across different situations, we never found an optimal strategy with values of the P^*(s,t) matrix differing from 0 or 1, indicating that males either always rejected or always accepted females of given qualities according to s and t. Figure 4 shows optimal strategies at three different sex-ratios (presented as the proportion of males in the population). Males were choosy when sex ratio was female biased (SR = 0.4, figure 4a) or balanced (SR = 0.5, figure 4b) but they paired at random when sex ratio was male-biased (SR = 0.6, figure 4c). Male mate choice depended on the interaction between both criteria of female quality. They tended to pair with female far from reproduction only when those were large enough to compensate for the opportunity cost associated with long lasting precopula (figure 4a, b). More precisely, let us consider a male using the mate choice strategy P^*(s, t). If he encounters a female, he already paid a searching time cost $1/\lambda$ and he has to decide whether to pair or to reject her. If he pairs with her, he will have to guard her for a time t before copulating with her and gain a fecundity f(s). If he rejects her, he returns to searching for a new female again. During the same time t, he will thus have a rate of gain that equals γ^*t. As a consequence the fitness gain he will achieve if he accepts the female is W_{accept} = f(s) whereas if he rejects her he will achieve a fitness gain of W_{reject} = γ^*t. Therefore, given γ^*, males using P^*(s, t) should accept females if f(s) > γ^*t. From this inequation, we can deduce the equation of the curve figuring the separation between accepted and rejected females given γ^*:

$$s = \frac{(\gamma^* t - 1)^{1/b}}{a} \tag{10}$$

Equation 10 suggests that given a particular value of t, male mate choice threshold on female size should vary according to the two parameters a and b describing the link between female size and fecundity. In the next section, we will investigate the role of female fecundity on male mate choice strategies and on the duration of mate guarding predicted in natural populations.
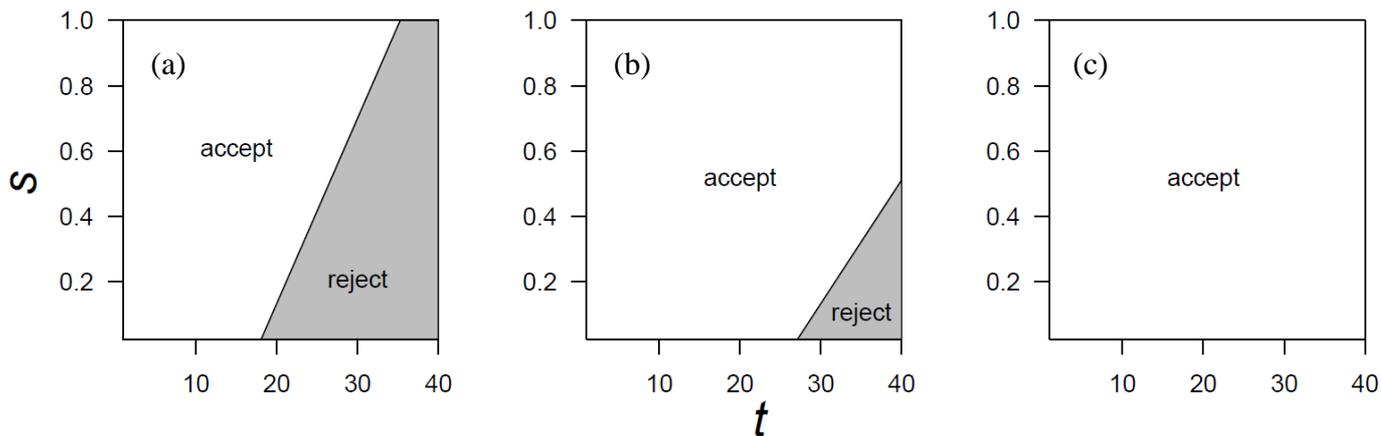
Figure 4: optimal mate choice strategies as a function of female body size and time left to reproduction. A female whose quality is comprised in the white zone is always accepted as mate by males. If on the other hand, the female's quality is in the grey zone, she should always be rejected as mate. Optimal mate choices are presented for three values of sex-ratio: (a) 0.4, (b) 0.5, (c) 0.6. Other parameters: $a = 1$, $b = 1$, $\lambda = 0.1$.

1.3.2 Effect of parameters of female's fecundity on optimal mate choice strategy

Fig. 5 shows the effect of the two parameters of female fecundity on mate guarding duration. Here, male mate choice on t depended also on s. Therefore, contrary to previous models of mate guarding, we could not predict a particular threshold of t below which males should choose females. In order to quantify the guarding duration predicted under male mate choice, we measured the mean proportion of female's reproductive cycle during which precopula occurred (hereafter, the mean proportion of guarding). This provided us with values that could not exceed 0.5, for which females are taken in precopula as soon as they start a new reproductive cycle. Under female biased sex ratio, males were choosy and the mean proportion of guarding varied with both parameters of fecundity (figure 5a). When a and b were small, it did not pay males to discriminate females on s because females varied little in fecundity within the population. As a consequence, males only tended to reject female far from reproduction, regardless of their size which resulted in rather short lasting precopula (figure 5a). When a and b increased, only large females carried several eggs in the population. Among these females, there was a large variation in fecundity and it paid males to become

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choosier on body size to reject small, less fecund, females. As a consequence, the mean proportion of guarding tended to increase in the population because males did not discriminate females on t as much as before (figure 5a). They almost never rejected large females, even when they were far from reproduction. On the other hand, they almost always rejected small, less fecund, females even when they were fairly close to reproduction. Under balanced sex-ratio, when a and b were small, males were barely choosy on either criterion (figure 4b, figure 5b). When b and/or a increased, males tended to increase their choosiness on s. A greater number of females were rejected when far from reproduction, therefore leading to lower mean proportion of guarding during female reproduction cycle (figure 5b). In certain cases (e.g. $a = 1.6$, $b = 8$, figure 5b) it was even similar to values observed for female biased sex-ratio.

1.3.3 Effect of encounter rate of females on optimal mate choice strategy

When λ increased, it decreased the time cost of searching for males because they were more likely to encounter an unpaired female rapidly. Fig. 6 shows the relationship between λ and males choosiness (defined as the proportion of rejected females) for female-biased (figure 6a) and even sex-ratio (figure 6b). As expected, increasing λ tended to increase male choosiness. However, for both sex-ratios, choosiness reached a plateau at $\lambda = 0.01$ after which it did not increase again. For female-biased sex ratio (SR = 0.4), increasing choosiness led to shorter guarding in the population (figure 6a). However, once choosiness stabilized, increasing values of λ led to longer lasting guarding duration which eventually reached the mean guarding proportion of guarding observed when males were less choosy (figure 6a). This indicates that greater choosiness only leads to shorter guarding duration observed at the population level when mate encounter rate is low. For balanced sex-ratio, male increasing choosiness does not contribute to decrease guarding duration (Figure 6b). Instead, with increasing λ, males encountered more unpaired females. Unpaired females rather far from reproduction were therefore likely to be rapidly found and taken in precopula before they became closer to reproduction.
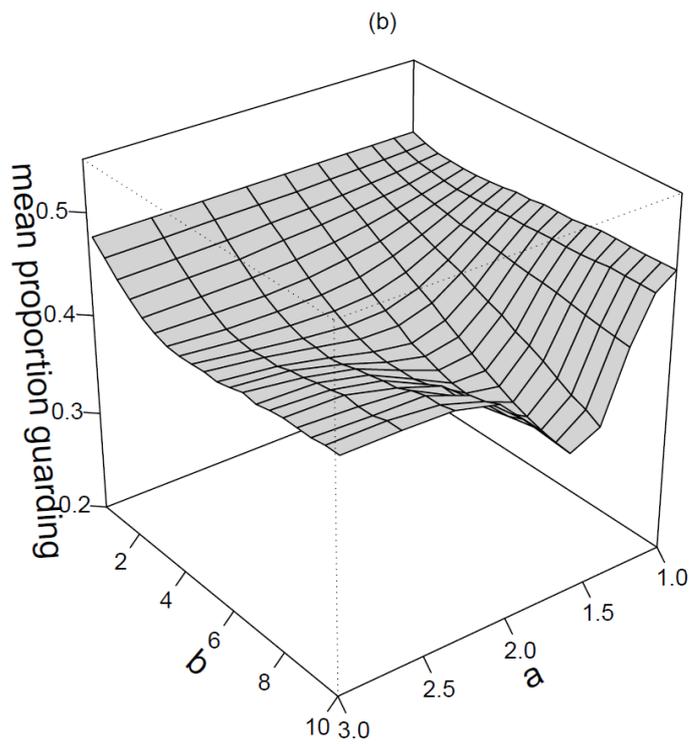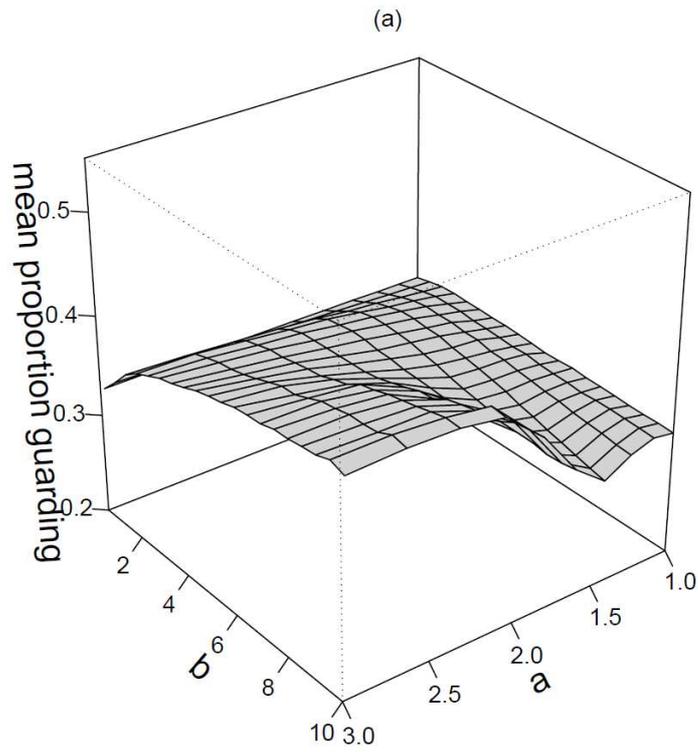
Figure 5: effect of parameters of fecundity a and b on the mean proportion of female reproductive cycle spent in precopula for (a) SR = 0.4 and (b) SR = 0.5. Other parameters: λ = 0.1.
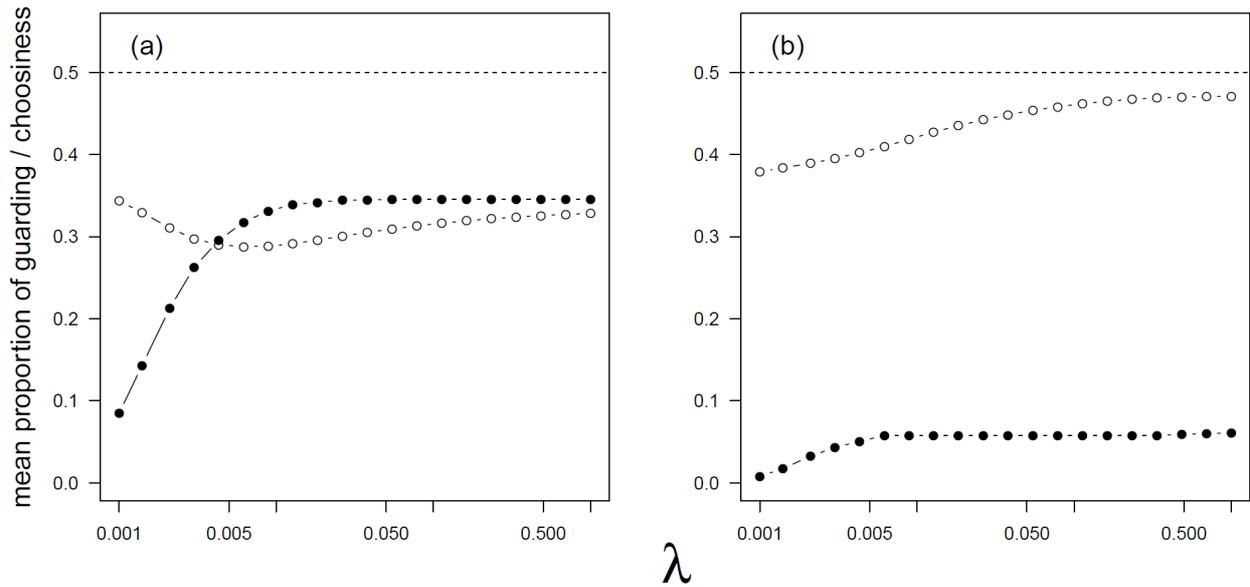
Figure 6: effect of mate encounter rate λ (logarithmic scale) on mean proportion of guarding during female's cycle (white dots) and on male choosiness (black dots) at (a) SR = 0.4 and (b) SR = 0.5. The horizontal dotted line figures the maximum mean proportion of guarding during female's cycle when males pair up at random and have a high encounter rate of females. Other parameters: $a = 1$, $b = 1$.

1.3.4 Mate choice on one or both criteria

Mean proportion of guarding during female's cycle changed if males tended to choose predominantly according to one or the other criterion of female quality. We ran the model considering that males did not have the possibility of discriminating females on s so that the optimal mate choice strategy $P*(t)$ corresponded to a threshold of $t*$ above which males rejected females. Under female-biased sex-ratio, when males discriminated females on the basis of t only, they tended to guard females closer to reproduction (i.e. lower $t*$) compared to situations where males chose on both criteria and females varied substantially in fecundity ($a = 1.6$, $b = 3$, figure 7). This resulted in shorter mean proportion of precopula during female's cycle observed at the population level (figure 7a). However, as soon as sex-ratio was even, males which only chose on t, where not choosy anymore and paired at random (figure 7b). If λ was large enough, this resulted in precopula lasting over the whole duration of female

reproductive cycle (figure 7a). On the other hand, when males chose on both cues, they were still choosy at an even sex-ratio (figure 7b), leading to slightly shorter guarding durations (figure 7a). Under male biased sex-ratio, it did not pay males to discriminate females anymore, except when those varied substantially in fecundity within the population ($a = 1.6$, $b = 3$, figure 7b). In that latter case, males still rejected some females before entering in precopula under strong competition for pairing, resulting in temporary mate guarding (figure 7a, b).
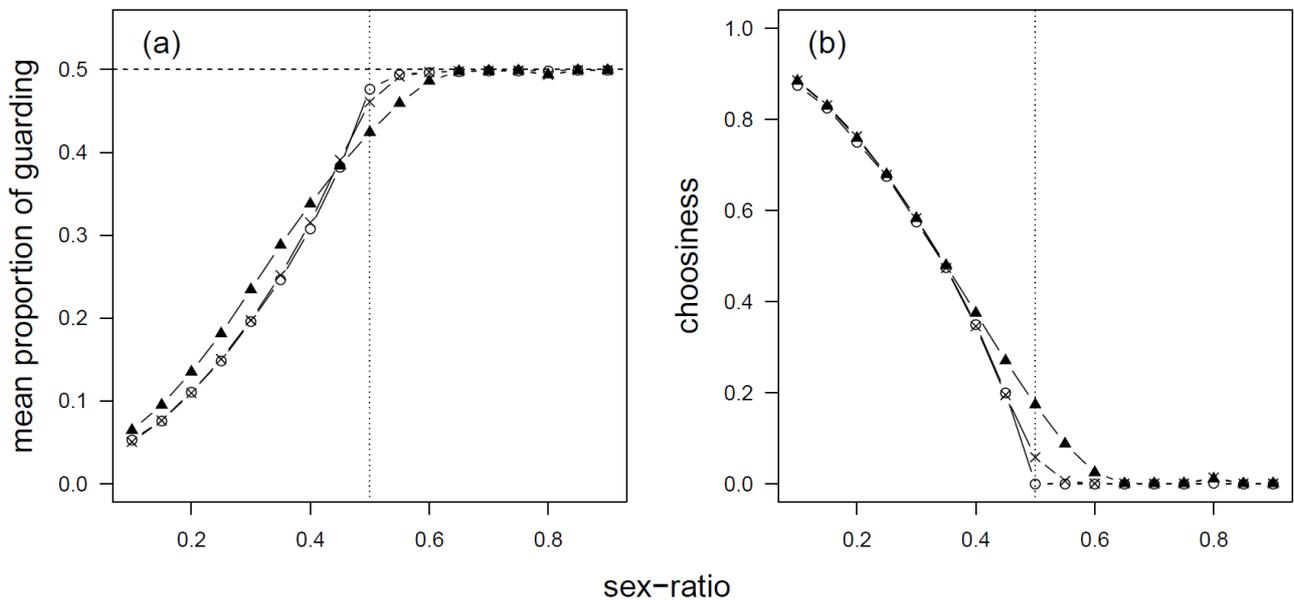
Figure 7: effect of sex-ratio on (a) the predicted mean proportion of guarding during female's cycle and (b) on male choosiness when males base their choice on t only (white dots) or both cues (crosses and triangles). Optimal choosiness on both cues was calculated for: (i) $a = 1$, $b = 1$, crosses, (ii) $a = 1.6$, $b = 3$, triangles. Vertical dotted lines figure balanced sex-ratio. The horizontal dotted line in (a) figures the mean proportion of guarding during female's cycle when males pair at random and have a high encounter rate of females. Other parameters: $\lambda = 0.1$.

1.3.5 Male mate choice and observed precopulatory mate guarding duration

Researchers observed a lot of variation in precopula duration in the field and they often relied on measures of mean precopula duration to infer processes that account for such variation (see table 1). They have mainly tried to explain temporary mate guarding under balanced sex ratio by considering difference in male competitive abilities and/or sexual conflict over guarding duration (e.g. Yamamura & Jormalainen 1996, Härdling et al. 2004). Here, we provided an alternative but not exclusive explanation, considering that males are capable of assessing female's body size as well as female's time left to reproduction before initiating precopula. Our main predictions are that:

(i) Under balanced sex-ratio, mean precopula duration should be shorter when females vary a lot in fecundity within the population. This is especially true when an important proportion of smaller females are little or even not fecund at all. In that case, it pays male to be choosier on female's body size before initiating precopula in order to avoid spending a lot of time guarding a small female associated with low fertility. In mate guarding crustaceans, this situation can exist if, for instance, males are likely to encounter immature smaller females while looking for a mate. Also, depending on species, the relashionship between female body size and fecundity varies (Sutcliffe 1993). Female disparity in fecundity within populations has been described to vary between species. It ranges from a twofold difference between the smallest and the largest female in the population, to about a hundredfold difference. In that latter case, smaller females carry almost no eggs while largest ones produce about a hundred eggs (Sutcliffe 1993).

(ii) Mean observed guarding duration should be shortened when encounter rate between partners is low. Under low density for example, males do not encounter potential partners often. Females who had just been released from a previous precopula and who are at the beginning of a new reproductive cycle may come closer to their next reproduction without pairing. This would tend to lead to temporary precopula observed at the population level. This is true even in cases where males are not choosy and are willing to pair with any females, even those far from reproduction. Mate density can be low due to dispersion of individuals in the environment or to the dilution of acceptable mates in the population. This latter case can occur in mate guarding crustaceans where several females are not breeding at a particular time and do not produce eggs. For instance, several

females could be performing growth moults at a given time, making more difficult for a male to find a suitable partner. Similarly, when females are castrated due to parasite infection, males tend to avoid them as mates (Bollache et al. 2002). Under high parasitic prevalence, the number of healthy fecund females should be low. Although males may not be choosy on either s or t, we predict rather short guarding duration because females available for pairing are difficult to find and are likely to come closer to reproduction before pairing up.

(iii) In accordance with previous models, we found precopulatory mate guarding duration to be strongly influenced by sex-ratio (Grafen & Ridley 1983, Härdling et al. 2004). Males tended to be choosier, pairing with females closer to reproduction and/or larger when sex-ratio was female-biased. Female biased sex-ratios are described in certain mate guarding crustacean species where individuals are infected with sex-ratio disorder microsporidian parasites (Terry et al. 2004). However, little is known about the fecundity of males becoming female under parasite infection and its effect on male mate choice (but see Hatcher & Dunn 1997 for a discussion). On the other hand, when sex-ratio was male biased, the strong male-male competition for access to females prevented males to be choosy. They rather paired with the first encountered female regardless of her quality. This is yet not entirely true in cases where females varied substantially in fecundity within a population. It was then possible to observe male choosiness under balanced or male-biased sex-ratios which resulted in temporary precopula at the population level.

1.4 Possible explanation for the disparity in findings between studies of male mate choice in mate guarding crustaceans

Our model can also help to understand why no consensus exists regarding criteria of female's quality used by males for mate choice (table 2). Depending on parameters of female's fecundity or sex-ratio, males could either base their choice on body size or on time left to reproduction. Fig. 8 presents three possible male optimal mate choice strategies under different conditions. At an even sex-ratio, when males encounter females sequentially, we predicted that males should not be strongly choosy if females in the sampled population do not vary markedly in fecundity (figure 7b, red line figure 8). However, in experiments, males are usually in presence of two females simultaneously. This may simulate a female-biased

sex-ratio leading the focal male to become choosier. Let us consider such an experiment using individuals originating from a population where females do not markedly vary in fecundity. Male mate choice strategy under this condition should correspond to the black line presented in Fig. 8. An experimenter aiming to test for the presence of a male mate choice on both cues would either present a male with two females of the same s but different t (A vs B or C vs D, figure 8) or with two females of the same t but different s (A vs C or B vs D, figure 8). In the former situation, males would tend to discriminate between females and choose the one with the smallest t. However, when facing females A vs C, both close to reproduction, males would tend to pair at random. When facing females B vs D, both far from reproduction, he would not pair at all. Although male mate choice strategy is based on both cues of female quality, such an experiment is more likely to detect a choice solely based on female's time left to reproduction. The exact contrary happens if females of the sampled population vary substantially in fecundity. In that case, male optimal mate choice strategy corresponds to the green line in Fig.8. When facing female A vs C or B vs D (figure 8), males would tend to pair up with the largest of the two. However, males would pair randomly when housed with two large females (A vs B, figure 8) and would reject both females when housed with two small females (C vs D, figure 8).

This may explain the measured difference in male mate choice strategies between mate guarding crustacean species (table 2). A wide variation in environmental conditions may act on mate choice making males more eager to discriminate females on one criterion or the other. However, experimental procedures involving one male simultaneously encountering two unpaired females may not reflect male choosiness in natural conditions. Under strong competition for females, males should accept almost every potential female and are likely to pair with the first one they encounter.

1.5 Model conclusion

Although our model predicts temporary precopula under balanced sex-ratio and specific relationship between female's body size and fecundity, male choosiness was never really high. Most of the time, males are predicted to only reject a small proportion of low quality females before entering in precopula. This calls into question the existence of male mate choice occurring before precopula when males encounter females sequentially. Alternatively, males could use different strategies of mate choice and mate sampling that

enable them to cope with the strong competition over pairing. In the next section, I will present an experiment testing male mate choice in mate guarding crustaceans when it occurs while males are already paired.
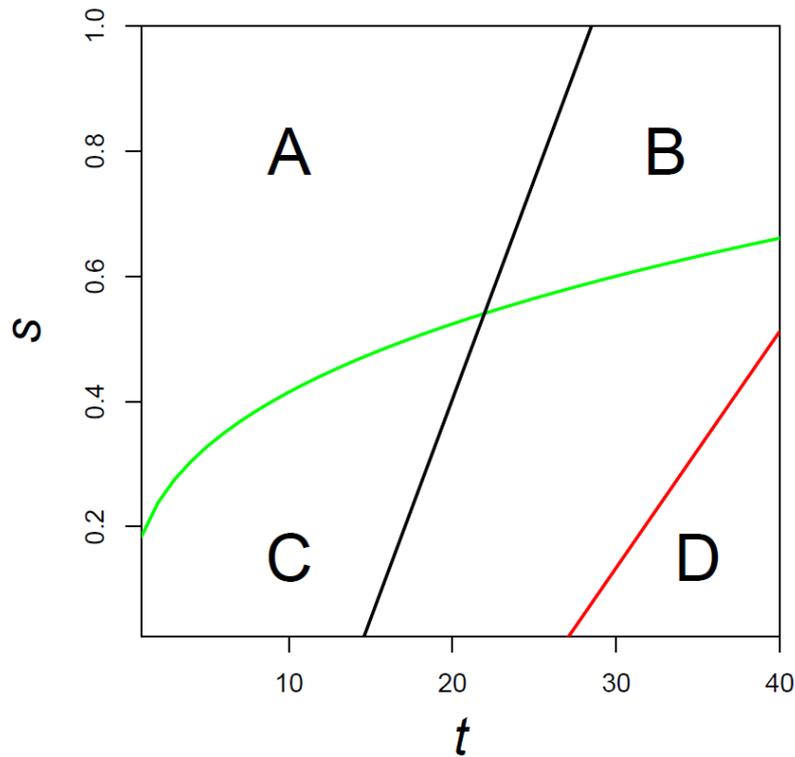
Figure 8: Optimal male mate choice strategies as a function of female's body size and time left to reproduction under three conditions: (i) SR = 0.5, $a = 1$, $b = 1$, red line, (ii) SR = 0.35, $a = 1$, $b = 1$, black line, (iii) SR = 0.4, $a = 10$, $b = 3$, green line. Different letters (A, B, C and D) represent potential females mate presented to males in simultaneous mate choice experiments. See the text for interpretation of the figure regarding potential biases associated with such experimental procedures. Other parameters: $\lambda = 0.1$.

2. Male mate choice during precopula

In mate guarding crustaceans, a few studies have showed that paired males are capable of releasing the female they currently guard to pair with a new one (Dick & Elwood 1989, Dick 1992, Iribarne et al. 1996, Wada et al. 2011). In the hermit crab *Pagurus minddendorffii*, males guard females by grasping the rim of their shell before spawning (Wada et al. 1996). However, when an unpaired female is at proximity of a couple, the paired male has been showed to sometimes assess the unpaired female and eventually leave his current partner to pair with her (Wada et al. 2011). This behaviour has also been observed in *G. pulex* where males can even simultaneously pair with two females, holding them perpendicularly relative to their own body for a few seconds before releasing one of them (Dick 1992, figure 9). This behaviour has been tested in a context of mate choice (Dick 1992, Wada et al. 2011). Authors showed that males tended to switch females when the unpaired female was relatively larger than the female they currently paired with. However, little is known about the role of female time left to reproduction in male switching decision. In addition, previous studies have only observed a few switching in controlled laboratory conditions (4 switching in Dick 1992 and 2 in Wada et al. 2011) suggesting that male decision rule may be subject to constraints. In manuscript 1, we studied mate switching in *G. pulex* by presenting paired males with unpaired females of various qualities and counting the number of trials were males were found to have changed females after 24h. Contrary to previous studies males did not seem to switch females when the new female was of relative better quality. Instead, we found that males switched females more often when the female they were initially paired with was of absolute low quality. Therefore, male decision rule did not seem to be based on the totality of the information available. Leaving his current female when she was of low quality regardless of the quality of unpaired females at proximity, males did not end up pairing with the best available female in every situation. We suggest that such a rule of thumb allows males to perform well in general if potential mates assessment is somehow constraints and/or subject to errors (McNamara & Houston 2009).

Contrary to what previously thought, precopulatory mate guarding is not a "passive phase" (Parker 1970). Here, we propose that precopulatory mate guarding may function as a sampling process by which males with poor information about the quality of females they initially pair with could improve their assessment. Under this hypothesis, unpaired males would first pair at random with the first female they encounter because high levels of male-

male competition prevent them from being choosy. It is only after they are paired that males could exert a mate choice and change partner when their current female is of low quality. This sampling process may exist in several species in which there is a substantial delay between mate encounter and possible reproduction. In birds species for instance, females have been found to change partner during the course of a season (Otter & Ratcliffe 1996, Ramsay et al. 2000, Jacot et al. 2010). Contrary to what we showed in *G. pulex*, during these "within-season" divorces, females left their current partner to pair with higher ranking newly widowed males (Otter & Ratcliffe 1996). Further studies are needed to understand the adaptive significance of using partial information in decision making related to mate choice under strong competition.

3. Conclusion

Even though long lasting precopulatory mate guarding are associated with high opportunity costs for males, it is difficult to find situations were males become highly choosy on female body size before entering in precopula. Only when females vary substantially in fecundity within a population should male reject small females in favour of larger ones. Even in that case, males should reject less than half the female they meet before entering in precopula which proves the difficulty for mate choice to arise from highly competitive situations (Barry & Kokko 2010). Alternatively, it is possible that males use different tactics of mate choice. Mating preferences sometime depend on individuals own quality (Riebel et al. 2010) or past mating experiences (Bleu et al. 2012). Because they are not shared between males, these preferences may therefore be less subject to competition than preferences shared with competitors. Also, males could exert mate choice during precopula. After having paired at random with the first female they contacted, they could switch partner before copulation. Such mate choice strategy is of particular importance when making inference about the mating pattern. Considering that males are capable of switching mates, punctual observation of pairs in natural populations may not necessarily inform about the mating pattern. In the next chapter, we will consider the link between mate choice strategies and mating patterns in species with precopulatory mate guarding.
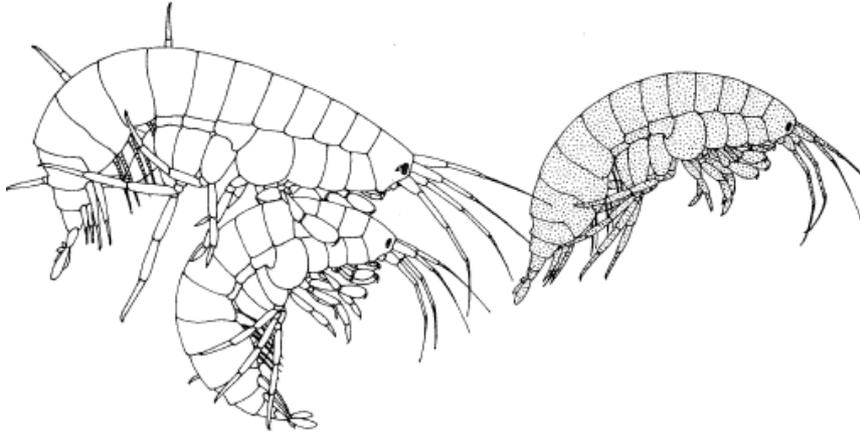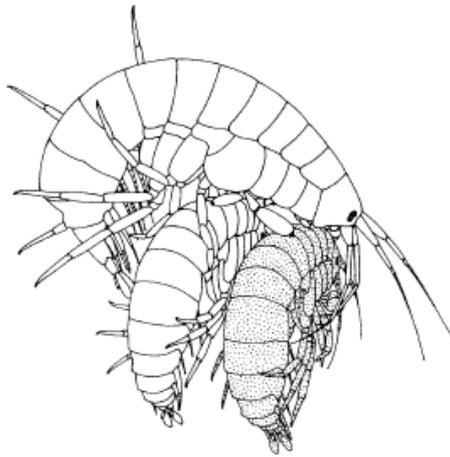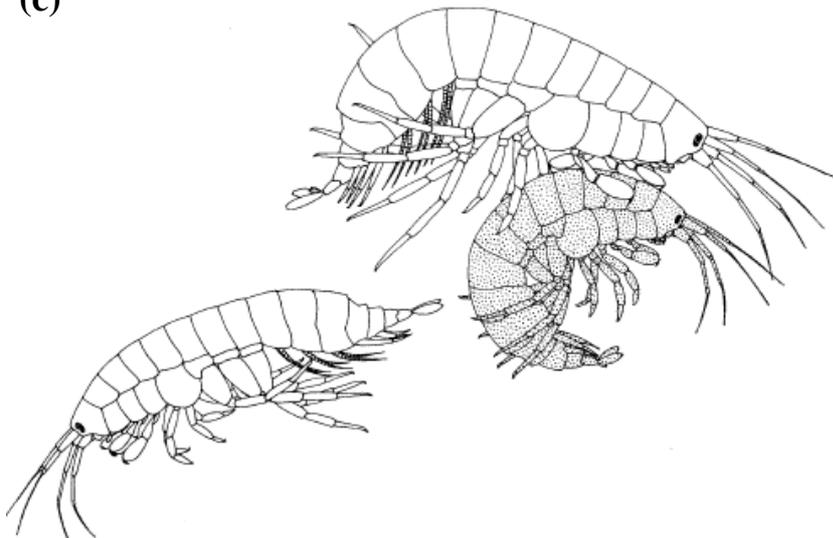
Figure 9: simultaneous manipulation of two females by a male *Gammarus pulex*. (a) An unpaired female comes at proximity to a couple. (b) The male grabs the two females simultaneously. (c) He releases one of the two female to pair with the other. From Dick 1992

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Simple decision rule for mate-switching during precopula in an amphipod crustacean. In prep

Simple decision rule for mate-switching during precopula in an amphipod crustacean.

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Abstract:

Comparable evaluation of potential partners is difficult when individuals encounter them sequentially. In addition, mate's quality is usually based on multiple traits which potentially impair their precise valuation before during mate choice. Under such constraints on decision making, it has been suggested that individuals could use simple decision rules that allow rapid and adaptive decision making. In amphipods, males guard females for a long time before copulation in response to strong competition for pairing. Consequently, mate discrimination prior guarding when females are encountered sequentially hardly evolves. However, during guarding, males have been described to switch females, leaving the female they guard for a new one. Although this behaviour potentially provides males with the possibility of comparing both females quality, little is known about male's decision rule for mate choice in such a situation. We measured switching probability when males were housed with two females differing in quality, one of which they were paired with. Female's quality was based on two criteria, their body size and their time remaining to copulation. Males made their switching decision solely on the basis of their current female quality. They had a greater probability to switch females when they were paired with a female of low absolute quality. This shows that males' decision rule was based on only a subset of the information available. We discuss this apparent maladaptive behaviour with respect to current theory on adapative decision making.

Introduction:

Mate choice is favored in population where mates greatly vary in quality (Parker 1983). It then pays individuals to discriminate among potential partners and show preference for those associated with the best direct or indirect fitness benefits. In addition, potential mates are generally dispersed in the environment. To spot their preferred partner within a population, animals have evolved sampling strategies because they do not have the capacity to encounter every potential partner before making a choice (Janetos 1980, Real 1990, Luttbeg 1996). When mates are encountered simultaneously, direct comparison of their relative quality is presumably facilitated (Bateson & Healy 2005). However, except in rare cases (e.g. leks), potential partners are encountered sequentially making their relative comparison difficult (Baker & Milinski 1991, Barry & Kokko 2010). In sequential sampling, choosy individuals have to assess each encountered potential mate quality before deciding whether to pair with it or to reject it (Luttbeg 1996). Because individual's quality is usually based on several modalities, they also potentially have to base their decisions on the assessment of multiple cues (Candolin 2003). When scramble competition for access to preferred mates is strong, individuals are yet less likely to find unpaired potential mates. In that context, explaining how they could afford to reject them is challenging (Barry & Kokko 2010).

Encountering, valuating and remembering the quality of different potential mates based on several cues in order to make rapid decisions in face of strong competition over mating may require cognitive capacity that animals sometime do not possess (Fawcett et al. in press). These constraints on mate sampling and quality assessment have led certain authors to suggest that animals may use simple decision rules to respond rapidly and adaptively to complex choice situations (Todd & Gigerenzer 2001, McNamara & Houston 2009, Fawcett et al. in press). For instance, they may assess mate's quality on one single cue (i.e. "take the best" heuristic, Hutchinson & Gigerenzer 2005) or sample a limited number of individuals before making a choice (i.e. best-of-n decision rule, Janetos 1980, Real 1990).

In mate guarding crustaceans, reproduction is tightly linked to females' molting cycle as they are only receptive for copulation shortly after molting and only for a few hours. Female fecundity is dependent on their body size with larger females carrying more eggs than smaller ones (Sutcliffe 1992). Within populations, molts occur with no synchrony and interval between two successive molts is longer for larger individuals. Females therefore greatly vary in both fecundity and their proximity to reproduction. In such mating systems, females receptive for copulation are scarce and scramble competition among males is strong. In

response to this competition, males engage in a precopulatory mate guarding (also called precopula or amplexus), holding on a female for a few hours up to several days before she molts and copulation occurs (Parker 1974, Jormalainen 1998). In simultaneous encounters males seem to discriminate females before entering in precopula. Laboratory experiments reported that they based their choice on female body size preferring larger, more fecund females over smaller ones (Reading & Blackwell 2007, Franceschi et al. 2010, Wada et al. 2011). They can also discriminate females according to their time left to molt (TLM) choosing preferentially the female closest to molt when presented to two unpaired females (Birkhead & Clarkson 1980, Thomson & Manning 1981, Dunn 1998, Lemaître et al. 2009). Choosing females close to molt (i.e. close to copulation) potentially provides males with a higher mating rate and shorter costly precopula (Jormalainen 1998). However, according to theory, males should not exclusively choose females according to one or the other cue. Rather, they are often supposed to discriminate partner by combining both source of information, preferring to consort with females that are the most fecund given the time they need to be held before copulation (Thomson & Manning 1981, Elwood et al. 1987).

Precopula can last several hours up to several days depending on the species (Jormalainen 1998). Despite this substantial time between mate encounter and copulation, males have often been assumed to be resolute in their choice, holding on tightly to their female against competitors and waiting for copulation. However, males have sometimes been observed to switch partner during precopula, releasing their current female to pair with a new one (Dick 1992, Iribarne et al. 1996, Wada et al. 2011). This mating behavior has received only little attention (but see Wada et al. 2011), although it would presumably be an efficient mate sampling process when simultaneous encounters of several unpaired potential partners before precopula initiation are rare. We studied male decision rule for mate switching strategy in *Gammarus pulex*, an amphipod crustacean. Our aim was to understand which modalities of female quality were involved in switching decision when a paired male encounters an unpaired female.

Material and methods:

Using the kick sampling method (Hynes 1954) and a hand net, sexually mature gammarids were collected in the river Suzon (Burgundy, France, N: 47°24,215′; E: 4°52,974′) between March and May 2010. Individuals were immediately brought back to the laboratory and housed in a large tank filled with well aerated water at 15°C that had been previously filtered

and UV treated for pathogens. For experiments, we directly collected gammarids from the stock tank. They were first gently separated from their current partner before being housed in glass cups also filled with UV treated water. Individuals used in experiments spent less than a week in the lab.

Males were first isolated in glass cups for 24h and fed with elm leaves ad libitum for acclimatization. After 24h, the leaves were removed and a female randomly chosen from the previously paired females was added to the cup to allow precopula formation (hereafter called the current female). Once the couple formed, which typically took a few minutes, we waited 20 minutes before adding a second single female randomly chosen from the previously paired females (hereafter called the new female). We counted the number of palpation attempts the male made towards the new female with his posterior gnathopods for 30 minutes. The three gammarids were then left to interact for 24h, after which we determined whether the male had changed partner for the new female. The male was then removed from the cup. We estimated his body size using the length of his fourth coxal plate (Bollache & Cézilly 2004) to control for its potential effect on male capacity to have access to partners (Fawcett & Johnstone 2003, Hardling & Kokko 2005).

Females in each trial were of different quality. We aimed to present the male with a new female of equal or better quality compared to his current female in an attempt to favor situations of mate switching. Before adding them to the cups, we roughly assessed females' relative quality on the basis of their body size and/or their TLM. The two females were considered varying in body size when we could observe a size difference with the naked eye. We estimated their TLM thanks to the maturity of embryos in their brood pouch (Geffard et al. 2010). Female were considered close to molt when carrying bright orange young in their brood pouch and far from molt otherwise. After experiments, we precisely assessed the quality of each female used in trials. To measure their TLM, we individually housed them with a new male until their molt to avoid biases related to female plasticity in molting time when unpaired (Galipaud et al. 2011). We then measured their body size following the same procedure used for males (see above). This provided us with 93 trials covering a wide spectrum of situations where the new female was either of better quality according to both cues, of better quality only according to size, of better quality only according to time left to molt, of same quality, or in a few cases, of worst quality according to both cues (in 4 out of 93 cases). On average, the new female was 0.22 mm ± s.d 0.33 larger and 6.21 days ± s.d 11.6 closer to molt than the current female. The mean size of current females was 1.87 mm ± s.d

0.22 and that of new females was 2.09 mm ± s.d 0.25. The mean TLM of current females was 10.17 days ± s.d 10.36 and that of new females was 3.96 days ± s.d 4.5.

Using other gammarids, we also allowed 53 males to form precopula with randomly chosen females. However, we did not add a new female to the cup. We recorded the number of split couples after 24h, therefore measuring the basal rate of couple separation.

Data analysis:

Before making a decision, males could have assessed one or the other female absolute quality (body size and TLM) or could have compared the two females. In both cases they may have assessed females' body size or TLM or a combination of these cues (Thomson & Manning 1981, Elwood et al. 1987). We therefore considered two categories of explanatory variables. (i) Simple variables were based on the absolute value of TLM and body size for both females. (ii) Composite variables included specific associations between simple variables. For this second category, we considered the difference variables: the values of the size difference ($D_s = S_n\text{-}S_c$) and TLM difference ($D_{tlm} = TLM_c\text{-}TLM_n$) between the two females. We also considered the ratio variables: values of the ratio of size over TLM for both females (R_c and R_n) and values of the difference between these ratios ($DR = R_c\text{-}R_n$). We did not have *a priori* knowledge about the relative importance of these different variables. Therefore, inferences about male mate choice behavior depended on a wide range of alternative models that included either simple or composite explanatory variables.

Analysis of males' number of palpation attempts or males' mate switching probability were performed separately. We compared alternative linear models generalized for a zero inflated negative binomial distribution in order to explain the number of palpation males did towards new females (R package "glmmADMB"). The probability of switching was studied by comparing logistic regression models. For both analyses we first constructed a set of candidate models including only biologically meaningful variables based on our expertise on gammarid biology. We then used AIC_c to identify best models that explains male's behavior (Burnham and Anderson 2002, Symonds & Moussalli 2011). We performed distinct AIC_c model selection procedures for set of models that included either simple or composite variables in order to avoid problems related to collinearity (Freckleton 2011). We calculated the difference ΔAIC_c between the model with the minimal AIC_c value (i.e. the best model) and alternative models. We also calculated for each alternative model its Akaike weights w_i as a measure of the weight of evidence that the model i is the best model to describe male's

behavior. For interpretations, we used a confidence set of models for which their cumulated weights equals 0.95. Each variable's importance within this set of models was assessed using a model-averaging method (Burnham & Anderson 2002, Symonds & Moussalli 2011). Variables with highest model averaged weights were expected to be of higher relative importance to explain male's behavior. Every analysis was conducted using R-2.15 (R Development Core Team 2012).

Results:

From the 122 trials we started with, 112 males initiated precopula with the first female (i.e. the current female). This represents 8% of mate rejection when both males and females were unpaired. In 19 of the 112 remaining trials, one of the two females died or was eaten by a male during the experiment. We thus used 93 trials for analysis.

Males' palpations towards the new female

During the first 30 minutes after the introduction of the second female in the arena, we observed 80 males performing palpations performing towards the new female (mean number of palpations $4.62 \pm$ s.d 3.44). However, we only observed simultaneous manipulation of both females by the male in one replicate. Based on the model selection procedure, males' motivation to palp the new female was mainly explained by their own size and the quality of their current female (table 1). Males tended to perform more palpations when they were large and when their current female was large and close to molting (table 2). The difference is TLM and size between the two females also tended to influence the number of palpation attempts, although the TLM and size of the new female alone did not seem to affect it. However, the close values of AIC_c (table 1) between the best models for the simple variables ($AIC_c = 411.75$) and the composite difference variables ($AIC_c = 411.26$) analysis does not allow a clear rejection of one or the other effect to explain the number of palpations. Males may have displayed more palpations when the difference in females quality was low or only when their current female was of bad quality. Number of palpations had no effect on male's probability to switch females (logistic regression for a binomial distribution, $\chi^2 = 0.13$, $df = 1$, $P = 0.72$).

Mate switching

Although 89 out of 93 trials involved a new female of relative better quality compared to males' current female, males switched females only 26 times. Thirteen out of 47 males switched females when the new female was of better quality than the current female according to both cues. Nine out of 19 males switched females when the new female was of better quality according to size only. Four out of 23 males switched females when the new female was of better quality according to TLM only. In the 4 situations where the new female was of lower quality according to both cues, males never switched females. On the other hand, in trials involving only one male paired with one female, couples split up 3 out of 53 times.

Similar to analysis of the number of palpations, when using a criterion of $\Delta AIC_c < 2$ for model selection, mate switching probability was only explained by male's current female quality (table 3). The best model to explain mate switching (i.e the model with the lowest AIC_c value among all models considered in both simple and composite variables analyses) only included the ratio of the current female size over her TLM as an explanatory variable. The male switched females when his female was of low quality; i.e. her ratio of size over time left to molt was low. This result is consistent with the model averaging procedure that indicated R_c as the major variable to consider to explain mate switching (table 4). It also pointed out the importance of the TLM of the current female alone and the difference in females TLM as explanatory variables (table 4). Males seemed to switch females with a greater probability when their current female was far from molting or when the difference in females TLM was high. Current female size alone did not seem to influence mate switching (table 4) and, as for palpations, characteristics of the new female were of little explanatory power for switching behavior (table 4).

Discussion:

When given a choice, the majority of males remained with their current female even when the new female was both larger and closer to molt (i.e. which potentially corresponds to a greater fitness payoff). Males thus sometimes neglected the better available option. When they did switch females, their decision appeared to be based on the characteristics of their current partner only. This markedly differs from previous studies of mate switching in mate guarding crustaceans which showed that males tended to change partner for larger, more fecund female

(e.g. Dick 1992, Wada et al. 2011). We suggest two possible interpretations for this apparent suboptimal decision making.

First it is still possible that males actually compared both females quality, but because of constraints on assessment, they could not detect the difference in females' quality. Mate assessment and mate guarding decision in crustaceans have been described to proceed as a complex behavioral sequence during which males sometimes grab females and exert antennae palpations (Dick & Elwood 1989). While in precopula, it may be difficult for males to accurately assess the quality of a new female. With poor information about her quality, they may be prone to errors leading to suboptimal decision making.

A second interpretation could be that males did not compare the two females' quality and based their pairing decisions solely on their current female quality. In many situations, it has been reported that simple decision rules under which animals purposely neglect a part of the available information could perform well (Hutchinson & Gigerenzer 2005). This may especially be true when perfect information about the environment comes from several sources. Animals processing all the available information may sometimes make optimal decisions but may also be prone to errors leading to suboptimal behaviors associated with low fitness payoffs. On the other hand, when decisions are only based on a subset of the information available, animals make fewer errors in assessment. Although such decision rules do not lead to the choice of the best option in every situation, they allow a rather sure fitness payoff over time. In certain situations the mean payoff of such simple decision rules can be greater than a more elaborate decision based on the assessment of multiple sources of information (Gigerenzer 2008). Instead of using a different strategy for every situation, hence being prone to errors, individuals using such rules of thumbs make good choices in general (McNamara & Houston 2009). Knowing only their current female quality, *Gammarus* males could have decided to leave her when a novel single female was close to the couple. Further investigations are needed to understand the fitness consequences of using such rules of thumbs in mate switching. This also raises an important question: how males value their current female when they do not compare her quality with the quality of other potential mates (Bateson & Healy 2005)? One answer could be that mate valuation depends on past mating experience. Choosy individuals with initially no information about mates' quality distribution in the population can update their decision rule according to previous reproduction (i.e. Bayesian decision making, Jennions & Petrie 1997, McNamara et al. 2006). For instance, "trade-up" choices (Halliday 1983, Bleu et al. 2010), according to which individuals choose mates of similar or higher quality than their previous one, have been described in several

species (e.g. in fishes, Bakker & Milinski 1991; in insects, Bateman et al. 2001). Instead of assessing the new female's quality, mate guarding males could decide to leave their current female when she is of lower quality than a threshold depending on prior knowledge of mates' quality distribution. Bayesian decision making in a context of mate choice has for example been showed in another species of mate guarding crustacean, *Gammarus lawrencianus* (e.g. Hunte et al. 1985). Under this hypothesis, comparative choice happens between reproductive events rather than between several available options within a reproductive event. Males can switch partners without assessing the new female's quality but they have to acquire knowledge about the quality of the female they are currently paired with. Assessing ratio of female size over TLM can provide enough information about current female's quality in order to make a switching decision (Elwood et al. 1987). Engaging in precopula may facilitate such assessment when it requires some time to be accurate (Goshima et al. 1998).

Male mate choice in gammarids may proceed as guarding-switching sequences. A male could first pair with the first encountered female and gather information about her quality while guarding (Goshima et al. 1998). If single females are available, he can then decide to switch females based on his knowledge of his current mate's quality. This is of particular interest considering that precopulatory mate guarding has almost only been though as a male competitive strategy (Grafen & Ridley 1983, Jormalainen 1998), but never as a mate sampling strategy. Mate guarding could represent a way for males to sample and find good quality mates. Under strong scramble competition, males perform long lasting mate guarding because they encounter few single females. This is also of major importance when making inference about the mating pattern based on the pairing pattern. Mate guarding crustaceans are often found to pair in an assorted manner in nature, with larger males paired with larger females and smaller males paired with smaller females (Crespi 1989). When considering possibility of mate switching, observations of the pairing pattern do not necessarily account for the subsequent mating pattern (Galipaud et al. in press). This is important when studying the consequences of such assortative mating on gene flow and selection.

The sampling rule that we described presumably make situations of mate choice easier to evolve as male capacity to sample females do not impede male capacity to reproduce. Because the sampling process occurs while they are already paired, males would eventually have access to reproduction even if they do not find a better partner. More generally, studies of mate choice usually consider reproduction to directly follow mate encounter. We believe

that switching rules before copulation must be common in species where latency exists between mate encounter and actual reproduction. This may especially be the case in several species of crustaceans with precopulatory mate guarding, but it may also exists in insect, monogamous birds or mammals, for which reproduction sometimes comprise a mate guarding phase. We hope that this will stimulate future theoretical research on sampling rules used by males that allow them to exert a choice under strong competition. Future studies could also focus on individuals' decision rules for mate choice when mates are difficult to compare and vary on several traits. Special emphasis should be made on the adaptive value of rules of thumbs over several mating events.

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Table legends:

Table 1: number of palpations towards the new female as a function of variables of females' quality. Each model included a given association of variables and models with no variables only included an intercept. For each model, we considered its AIC_c value and their difference ΔAIC_c with the best model, that is, the model with the greatest weight (w_i).

Footnote: S_{male} size of male, S_c size of the current female, TLM_c time left to molt of the current female, S_n size of the new female, TLM_n time left to molt of the new female, R_c ratio of size over time left to molt for the current female, R_n ratio of size over time left to molt for the new female, D_{tlm} difference in time left to molt between the two females, D_s difference in size between the two females, DR difference in ratio of size over time left to molt between the two females.

Table 2: Model-averaged estimates for the effect of variables of female qualities on male number of palpations towards the new female. For each variable, we considered its averaged coefficient (β) and the standard error (adjusted SE) and 95% confidence interval for β.

Footnote: [a] For the model averaging analysis, we summed the weights of each models where the considered variable appeared based on the model selection process.

S_{male} size of male, S_c size of the current female, TLM_c time left to molt of the current female, S_n size of the new female, TLM_n time left to molt of the new female, R_c ratio of size over time left to molt for the current female, R_n ratio of size over time left to molt for the new female, D_{tlm} difference in time left to molt between the two females, D_s difference in size between the two females, DR difference in ratio of size over time left to molt between the two females.

Table3: probability of switching as a function of variables of females' quality. Same remarks than in table 1.

Table 4: Model-averaged estimates for the effect of the variables of female quality on male probability of switching females. Same remarks than in table 2.

Figure caption:

Figure 1: male probability of mate switching as a function of (a) the current female ratio of size over time left to molt (R_c) and (b) the current female time left to molt (TLM_c). Higher values of R_c and low values of TLM_c were associated with greater female quality. We added a jitter on the y axis values of data for representation purpose. This does not account for the real values that can only take 0, when the male stayed with his current female or 1 when he switched females. Solid curves represent the estimated logistic regression based on GLM model with a logit link function ($\chi^2 = 23.72$, $df = 1$, P < 0.001 for (a) and $\chi^2 = 18.38$, $df = 1$, P < 0.001 for (b)).

Table 1

Type of variables	Models					AICc	ΔAICc	w_i
Simple								
	S_{male}	S_c	TLM_c			411.75	0.00	0.21
	S_{male}	S_c	TLM_c	S_n		412.16	0.41	0.17
	S_{male}	S_c	TLM_c	S_n	TLM_n	413.88	2.13	0.07
	S_{male}	S_c	TLM_c		TLM_n	414.01	2.26	0.07
	S_{male}	S_c				414.2	2.45	0.06
	S_{male}	S_c		S_n		414.74	2.99	0.05
		S_c	TLM_c			414.79	3.04	0.05
	S_{male}		TLM_c			414.83	3.08	0.05
		S_c	TLM_c	S_n		415.2	3.45	0.04
	S_{male}					416	4.25	0.03
	S_{male}		TLM_c	S_n		416.02	4.27	0.03
	S_{male}	S_c		S_n	TLM_n	416.09	4.34	0.02
	S_{male}	S_c			TLM_n	416.24	4.49	0.02
		S_c				416.38	4.63	0.02
			TLM_c			416.90	5.15	0.02
		S_c		S_n		416.92	5.17	0.02
	S_{male}			S_n		417.08	5.33	0.01
	S_{male}		TLM_c		TLM_n	417.10	5.35	0.01
		S_c	TLM_c		TLM_n	417.12	5.37	0.01
	S_{male}	S_c	TLM_c		TLM_n	417.32	5.57	0.01
						417.55	5.8	0.01
			TLM_c	S_n		417.95	6.2	0.01
Composite								
	S_{male}					416	0.00	0.40
						417.55	1.55	0.18
	S_{male}	R_c				417.74	1.74	0.17
	S_{male}		R_n			418.26	2.26	0.13
		R_c				419.58	3.58	0.07
			R_n			419.72	3.73	0.06
	S_{male}	D_{tlm}	D_s			411.26	0.00	0.49
	S_{male}		D_s			412.82	1.55	0.22
		D_{tlm}	D_s			413.78	2.52	0.14
			D_s			414.89	3.62	0.08
	S_{male}	D_{tlm}				415.03	3.77	0.07
	S_{male}					416	0.00	0.55
						417.55	1.55	0.25
	S_{male}	DR				418.05	2.05	0.20

Table 2:

Type of variables	variables	$\sum w_i$ [a]	β	Adjusted SE	95% C.I for β
Simple variables					
	TLM_c	0.75	-0.016	0.01	-0.04 to -0.001
	TLM_n	0.23	-0.009	0.55	-0.17 to 0.09
	S_c	0.84	0.904	0.47	0.17 to 1.99
	S_n	0.44	-0.282	0.47	-1.56 to 0.27
	S_{male}	0.81	0.659	0.36	0.1 to 1.52
Composite variables					
	R_c	0.23	0.04	0.26	-0.35 to 0.68
	R_n	0.19	-0.0009	0.2	-0.4 to 0.39
	S_{male}	0.69	0.524	0.39	-0.0007 to 1.53
	D_{tlm}	0.7	-0.01	0.01	-0.04 to 0.0004
	D_s	0.93	-0.771	0.34	-0.16 to -1.5
	S_{male}	0.78	0.605	0.36	0.06 to 1.48
	DR	0.2	0.016	0.18	-0.27 to 0.43
	S_{male}	0.75	0.559	0.39	-0.01 to 1.51

Table 3

Type of variables	Models					AICc	ΔAICc	w_i
Simple								
		S_c	TLM_c			81.16	0.00	0.22
			TLM_c			81.30	0.14	0.20
	S_{male}		TLM_c			82.91	1.75	0.09
	S_{male}	S_c	TLM_c			83.00	1.84	0.09
		S_c	TLM_c		TLM_n	83.36	2.20	0.07
		S_c	TLM_c	S_n		83.36	2.20	0.07
			TLM_c	S_n		83.43	2.27	0.07
			TLM_c		TLM_n	83.45	2.29	0.07
	S_{male}		TLM_c	S_n		85.10	3.94	0.03
	S_{male}		TLM_c		TLM_n	85.12	3.96	0.03
	S_{male}	S_c	TLM_c		TLM_n	85.25	4.09	0.03
	S_{male}	S_c	TLM_c	S_n		85.26	4.10	0.03
Composite								
		R_c				78.40	0.00	0.57
	S_{male}	R_c				80.16	1.76	0.24
		R_c	R_n			80.54	2.14	0.19
		D_{tlm}				81.56	0.00	0.49
		D_{tlm}	D_s			82.49	0.93	0.31
	S_{male}	D_{tlm}				83.26	1.70	0.21
		DR				98.88	0.00	0.52
						100.02	1.14	0.29
	S_{male}	DR				100.85	1.97	0.19

Table 4

Type of variables	variables	$\sum w_i$ [a]	β	Adjusted SE	95% C.I for β
Simple variables					
	TLM_c	1	0.111	0.03	0.05 to 0.16
	TLM_n	0.20	0.001	0.09	-0.18 to 0.18
	S_c	0.51	0.981	1.37	-1.71 to 3.67
	S_n	0.20	0.031	0.65	-1.25 to 1.32
	S_{male}	0.29	-0.219	0.70	-1.59 to 1.15
Composite variables					
	R_c	1	-6.920	2.06	-11 to -2.88
	R_n	0.19	0.011	0.26	-0.49 to 0.52
	S_{male}	0.24	-2.489	0.6	-1.34 to 1.02
	D_{tlm}	1	0.112	0.03	0.05 to 0.16
	D_s	0.31	-0.327	1	-3 to 0.9
	S_{male}	0.21	-0.152	1.1	-2.9 to 1.43
	DR	0.71	-0.563	0.53	-1.60 to 0.47
	S_{male}	0.19	-0.082	0.47	-1 to 0.84

Figure 1
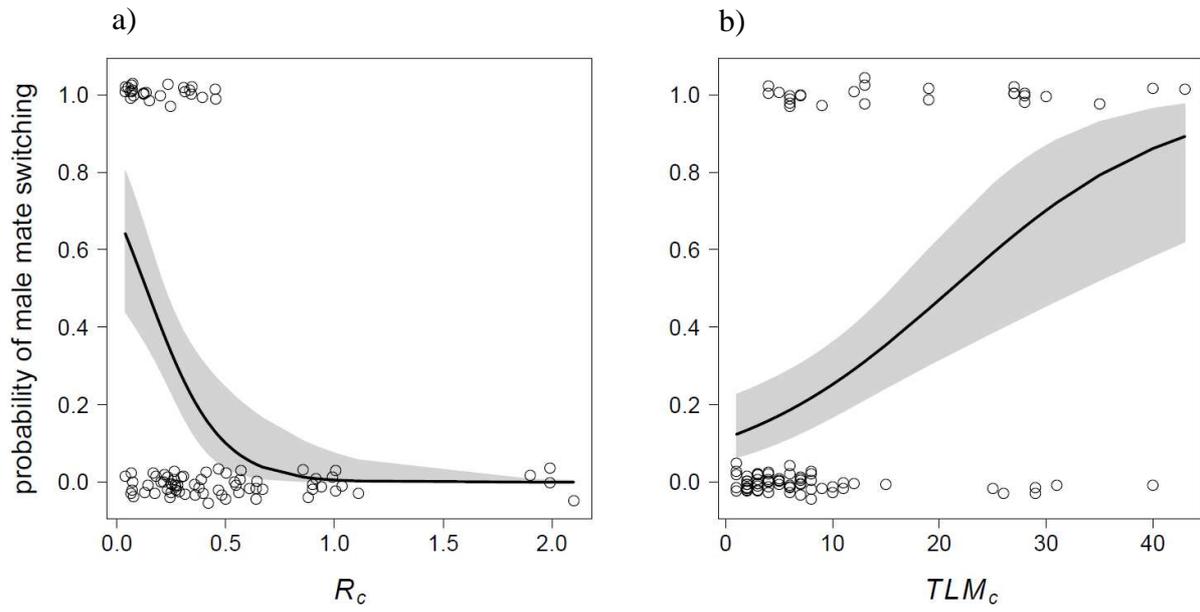
Chapter 3

Linking preferences to patterns: different hypotheses for the occurrence of size-assortative pairing in mate guarding crustaceans

Matthias Galipaud, Loïc Bollache, François-Xavier Dechaume-Moncharmont, Rémy Destrebecq, Clément Lagrue, Rémi Wattier, Zoé Gauthey

1. Causes for size-assortative pairing

Many hypotheses have been put forward to explain size-assortative pairing observed in natural population (Crespi 1989). Although certain possible causes have been partly disputed, none have been unambiguously shown to lead to homogamy. In the next section, I will present some of these hypotheses before presenting a new one susceptible to account for size-assortment among pairs.

1.1. The mechanical constraints hypothesis

Among first attempts to explain homogamy was the claim that individuals pairing with alike achieved a greater mating success. Pearson (1899) argued that because assorted couple

have greater fertility, men should seek women of similar size. Later, in an attempt to explain size-assortative pairing in crustaceans, Crozier and Snyder (1923) hypothesised that individuals were mechanically unable to pair or mate with partners differing too much in body size compared to their own. Mechanical constraints on mating can occur if there is an allometry between genitalia size and body size. Size incompatibilities in genital organs may impede mating between partners differing too much in size, hence leading to size-assortative mating at the population level. In leaf beetles *Trirhabda canadensis* for instance, size-assortative mating had been suggested to result from the inability of disparately sized individuals to successfully achieve intromission (Brown 1993). However, such mechanical constraints have been mainly considered to apply to species with hard exoskeleton rather than species with soft bodies (Willoughby & Pomerat 1932). Male's capacity to hold a female may also be subject to mechanical constraints. In water striders, males ride females on their back on the water surface. The efficacy of this grasping posture has been showed to be highly dependent on male and female relative body size within a pair, which would explain why they are generally found to be assorted by size (Han et al. 2010). In gammarids, small males have been thought to be unable to properly hold a too large female due to the small size of their dactyli compared to the female specialized sites present at the surface of her cuticle (Platvoet et al. 2006). Smaller males may then be disadvantaged in holding large females compared to larger males, hence leading to size-assortative pairing (Crozier & Snyder 1923). However, Birkhead & Clarkson (1980) showed that small males were actually able to initiate pairing with large females. Although this observation says little about the relative disadvantage of smaller males in holding large females for a long time, the mechanical constraints hypothesis is now rarely put forward to explain size-assortative pairing.

1.2. The loading constraints hypothesis

Field surveys in gammarids have revealed positive size-assortative pairing, which has not always been found in laboratory trials. Certain authors thus thought about possible environmental effects, such as current velocity on the occurrence of size assortment among pairs (Adams & Greenwood 1983). They hypothesized that loading constraints could explain size-assortative pairing because small males may be unable to efficiently swim in current while carrying a relatively larger and heavier female (Adams & Greenwood 1983, Greenwood & Adams 1984). Males therefore face a trade-off between pairing with larger, more fecund

females but difficult to carry in currents and pairing with smaller females easier to hold. Only males relatively larger than their female would be able to hold her. This would explain why pairs are size-assorted and why males evolved larger body sizes compared to females (Adams & Greenwood 1983, 1987, Adams et al. 1985, Greenwood & Adams 1984, 1987). This hypothesis has strongly been criticised by Ward (1986, 1987). Ward (1986) measured the strength of size-assortative pairing found in two populations of amphipods, one living in a pond, the other living in a stream. According to Adams and Greenwood's hypothesis, we would expect two main patterns: (i) size assortment should be stronger in the stream population because males should only pair with females which they are capable of holding in the current flow and (ii), the size ratio of male/female within pairs should be lower in the pond population compared to the stream population because males are not constrained by the current in the pond and can pair with relatively larger females. Contrary to these expectations, Ward (1986) found a higher size ratio of male/female for pairs in the pound compared to pairs in the stream (but see Greenwood & Adams 1987). On the other hand, although he did measure size-assortative pairing in both habitats, he did not compare these measures and he did not report 95% confidence intervals around the calculated Pearson's correlation coefficients. We applied the Fisher's z method to calculate confidence intervals around Ward's measures of size-assortative pairing. In the stream population, he measured size assortment among 983 pairs and found r = 0.47, CI [0.42; 0.52]. In the pond population, he measured size assortment among 229 pairs and found r = 0.29, CI [0.16; 0.40]. This means that in Ward's study, size-assortative pairing was actually lower in the pond population than in the stream population, in accordance with Adams and Greenwood's predictions. It is therefore still unclear whether the loading constraints hypothesis can account for size-assortative pairing. In fact the debate between Ward and Adams and Greenwood rapidly became orientated towards possible explanations for the observed sexual size dimorphism in amphipods (Greenwood & Adams 1987, Ward 1987). Nowadays, very few studies aiming to understand the causes of size-assortative pairing continue to put forward this hypothesis (but see Williams 2007).

1.3. The spatio-temporal size heterogeneity hypothesis

Let us consider a population where individuals pair randomly but different size classes of individuals occupy different habitats. If we measure assortment by taking every individual

regardless of their habitat for the analysis, we would expect size-assortative pairing to result from the spatial segregation of individuals according to their body size. In *G. pulex*, individuals of different sizes have been shown to inhabit different substrates in the river (Miller & Buikema 1977). Birkhead and Clarkson (1980) thus sampled gammarids in 9 different patches. They found that individuals significantly differ in body sizes between patches. They also found no size-assortative pairing occurring within each patch but a strong size assortment taking every sampled pairs regardless of their initial patch for the analysis. Although these results would argue in favour of a role of spatial size heterogeneity in the apparition of size-assortative pairing, subsequent studies have pointed out that Birkhead and Clarkson (1980) used a rather small sample size within each patch to measure it (typically n < 15). Birkhead and Clarkson found rather high values of Pearson's correlation coefficient in each patch (mean value among patches; r = 0.39). A priori power test reveals that with less than 25 sampled pairs, this rather strong correlation appears non-significant. When repeating their test by taking into account this possible bias, size-assortative pairing appeared to be statistically significant within samples (Ridley 1983, Thompson & Moule 1983). This shows that size-assortative pairing is likely to be caused by another mechanism than spatial heterogeneity of different size classes. In addition, Bollache et al. (2000) conducted a similar experiment by sampling gammarids in pairs from three different substrates in the river. Thanks to large sample size for each habitat they revealed that, although individuals from different substrate differed in body size, hence leading to strong overall size assortment among pairs (e.g. overall assortative pairing in Saulon-la-Rue, r = 0.93, CI [0.90; 0.95], Bollache et al. 2000), size-assortative pairing also occurred within pairs found in each substrate. Like Thompson and Moule (1983), they concluded that size-assortative pairing in *G. pulex* is likely to result from a different mechanism than spatial size heterogeneity.

Instead of individual of different size being segregated in space, they can be segregated in time. Individuals of different size can for instance have different breeding period or different duration of sexual receptivity leading potential partners of similar size to meet more frequently than expected at random (Crespi 1989). No studies but one seemed to find a situation where such mechanism leads to size-assortative mating. In the Orb-web spider *Nephila clavata*, precopulatory mate guarding occurs before females' final moult after which they are adult and receptive for copulation (Miyashita 1993). Miyashita (1994) measured size-assortative mating during the course of the season. He found that larger females moulted and became sexually receptive earlier in the season compared to smaller females. Similarly, larger males were found in precopula earlier in the season compared to smaller males. As a

consequence, across the season, positive size-assortative mating was found to occur although no size assortment within pairs occurred at any given day of the season (Miyashita 1994).

1.4. The sexual selection hypothesis

Although the different hypothesis I presented so far received reasonable amount of attention in the literature, mechanisms of sexual selection are surely the most invoked cause for size-assortative pairing. The sexual selection hypothesis suggests that size-assortative pairing results from males and females behaviours related to mate choice and/or competitiveness. Two main mechanisms have been put forward to explain it. The first one considers that in a population where only one sex is choosy and prefers larger mates, size-assortative pairing should arise if large individuals have an advantage over smaller one in getting access to preferred mates. The second one suggests that size-assortative pairing results from a mutual mate choice where both sexes prefer larger mates. In chapter 2, I already presented empirical evidence for male preference for large females in mate guarding crustaceans. On the other hand, very little is known about female mate choice in these species. In the following section, I will consider the sexual selection hypothesis for size-assortative pairing in mate guarding crustaceans. I will first present evidences for large male mating advantage before considering the possibility of female mate choice for large males in these species.

1.4.1. Large male competitive advantage

In their seminal paper about male competitive strategies in common toads, Davies & Halliday (1979) observed a surprising male behaviour. Facing strong scramble competition for access to females, unpaired males were able to displace other, already amplexed males from the back of their female partner in order to take their place. These takeovers have later been suggested to play a role in antagonistic competition occurring between males of mate guarding crustaceans. Takeovers have also been often put forward to explain size-assortative pairing because larger males are presumably better than smaller males at displaying them, hence getting access to larger preferred females (Birkhead & Clarkson 1980). In table 3, I reviewed different studies that tested the occurrence of takeovers in amphipods. Although takeovers have been suggested to occur in certain species, only two studies reported a large

male advantage at performing them. In addition, no study showed any evidence for males trying to takeover larger females. Overall, due to their low prevalence and the lack of evidence for their link with a mate choice for large females, it seems unlikely that takeovers alone account for the size-assortative pairing found in mate guarding crustaceans. It is also worth noting that no actual takeover behaviour have ever been observed in these studies. Experiments were usually conducted by housing one couple and one single male in a cup. Cups were then checked only once or twice a day for changes in the male guarding the female. Such observed changes are only indirect evidences for takeovers. Franceschi et al. (2010) performed the same kind of experiment except that they watched individuals' behaviour in cups for 30 minutes. Interestingly, they did not observe any takeovers but witnessed separations of couples probably due to frequent antagonistic interactions that single males did toward paired males. Separations occurred 10% of the time, which roughly corresponds to previously reported proportion of supposed takeovers (e.g. table 3, Birkhead & Clarkson 1980, Ward 1983, Elwood et al. 1987, but see dick & Elwood 1990, Sutherland et al. 2007).

Table 3: occurrence of takeovers in different species of amphipods. The value reported in the table represents the percentage of takeovers measured by housing one couple with one single male and looking for changes in the male guarding the female after a given time. Large males were considered to have a competitive advantage when authors found a significant positive correlation between male body size and the occurrence of takeovers. Similarly, males were considered to prefer larger females when authors found a significant correlation between female body size and the occurrence of takeovers.

Species	Percentage of takeovers	Large male advantage	Preference for large females	References
Gammarus pulex	10.7 %	No	No	Birkhead & Clarkson 1980
Gammarus pulex	9 %	Yes	No	Ward 1983
Gammarus pulex	15 %	Yes	No	Elwood et al. 1987
Gammarus pulex	0 %	No	No	Dick & Elwood 1990
Paracalliope fluviatilis	0 %	-	-	Sutherland et al. 2007
Hyalella azteca	33 %	-	No	Cothran 2008b
Gammarus pulex	0 %	-	-	Franceschi et al. 2010

Male competitiveness is not restricted to takeovers in mate guarding crustaceans. Larger unpaired males have often been observed to have an advantage over smaller ones in gaining access to unpaired females (Ward 1983, Elwood et al. 1987, Adams et al. 1989, Iribarne et al. 1996, Cothran 2008b, but see Jormalainen et al. 1992, Sutherland et al. 2007). Two studies also showed a male preference for larger females (Elwood et al. 1987, Iribarne et al. 1996), one study reported no male preference based on female body size (Adams et al. 1989) and two did not measure male mating preferences (Ward 1983, Cothran 2008b). Overall, it is not really clear whether, in mate guarding crustaceans, large males enjoy an advantage over smaller males in pairing with large females.

As an alternative to the hypothesis of a large male advantage in initiating precopula with large females, Elwood & Dick (1990) proposed that costs associated with mate guarding could explain the observed size-assortative pairing. More precisely, they suggested with a verbal model that the relative greater energy cost associated with guarding may generate variation among males in the capacity to undergo precopula. Even though they both prefer to pair with larger females, only larger and stronger males have the capacity to start to guard females early in their moulting cycle, making them unavailable for smaller males who would eventually pair up with smaller females. In that scenario, larger males have an advantage in guarding females, not in getting easier access to them and this should result in size-assortative pairing. According to this "timing hypothesis", size-assortative pairing results from the tendency of larger males to pair with females earlier in their moulting cycle than smaller males do. However, size-assortative pairing should not result from a direct advantage over smaller males in contest for access to females. This hypothesis has received several attentions. Authors have acknowledged that precopula is an energy-demanding behaviour (Robinson & Doyle 1985, Jormalainen et al. 2001, Sparkes et al. 2002, Plaistow et al. 2003), and that larger males were more tenacious in precopula (Ward 1983, Plaistow et al. 2003) and often guarded females for longer durations compared to smaller males (Ward 1984a, Elwood & Dick 1990, Hume et al. 2002). However, the timing hypothesis has been partially disputed by Hume et al. (2002) in an experiment which found size-assortative pairing in situations where both small and large females were close to moulting. In those situations, assortative pairing could therefore not result from a large male advantage in holding females for longer time than smaller males. Although the timing hypothesis is still a possible cause for size-assortative pairing, authors admitted that other form of male-male competition may play a role in creating such pattern.

1.4.2. Female mating preference for larger males

As introduced above, another possibility for size-assortative pairing to arise is that both males and females prefer larger mates (Parker 1983). Smaller individuals would therefore be rejected as mates by larger individuals, leading them to eventually pair up with each other (Johnstone et al. 1996). Although in mate guarding crustaceans, males seem to show a preference for larger, more fecund females, it is still not clear whether females prefer to mate with larger males. Mate guarding is a coercive male mating strategy so that female usually cannot provoke a male to pair with her. They have been described to sometimes resist males' attempts to initiate precopula (Jormalainen & Merilaita 1993, 1995, Sparkes et al. 2000, 2002). This resistance behaviour is often thought to be an adaptive response to the sexual conflict presumably occurring over guarding duration (Parker 1979). However, it has also been proposed to play a role in male discrimination (Ridley & Thompson 1979, Jormalainen & Merilaita 1993, 1995, Cothran 2008c, Cothran et al. 2012). Authors have argued that females may be more likely to be held in precopula by larger males because those are better at overcoming female's resistance to precopula attempts (Ridley & Thomson 1979, Jormalainen & Merilaita 1993, 1995). Smaller males would then presumably be less likely than larger males to pair with large preferred females, hence leading to size-assortative pairing.

Like previous ones, this last possible cause for size assortative pairing suffers from the lack of empirical studies actually reporting observations of the whole pairing process in natural populations. Many of aforementioned behaviours and mechanisms put forward to explain size assortment among pairs may be strongly constrained by scramble competition for mates. Tests of mating preferences generally involved one male having to choose between two females in a cup, a design that is subject to limitations (Wagner 1998, cf chapter 2). The theoretical approach presented in Chapter 2 tended to suggest that under balanced sex-ratio and sequential encounter of potential partners, males should barely be selective on female body size. Unfortunately, pairing processes are difficult to investigate in the field. An alternative can be to study pair formation theoretically, using computer simulations and *in silico* experiments. In the next section, I will present a new hypothesis for the occurrence of size-assortative mating in mate guarding crustaceans. This summarizes the work my colleague and I conducted in manuscript 2.

2. A new hypothesis for size-assortative pairing

It remains challenging to explain size-assortative pairing from a directional preference based on body size that occurs before entering in precopula (i.e. every male in the population prefers large females). Alternatively, size-assortative pairing could come from a male mate choice during precopula instead of before initiating in precopula. If every male prefers to switch for larger females but only large males are able to do so, large males will presumably tend to accumulate with larger females, even though they initially paired randomly. However, we showed in the previous chapter that male mate switching does not seem to be based on the quality of single females encountered during precopula in *G. pulex*. At least for this species, it seems therefore unlikely that such decision rule leads to a pattern of size-assortative pairing, although we could not rule out this possibility without studying the pairing process in natural conditions.

Apart from directional preferences for body size, other kinds of mating preferences have rarely been considered to explain size-assortative pairing in mate guarding crustaceans. Mating preferences can depend on individual's current quality or condition. Individuals of many species prefer to mate with partners of similar phenotypes (i.e. homotypic preferences, Burley 1983). In cichlid fish for instance, males have been suggested to prefer to consort with females of similar sizes, leading to a pattern of size-assortative mating (McKaye 1986). Individuals can also discriminate between partners according to a threshold of quality depending on their own condition (Riebel et al. 2010). This is the case if less competitive individuals display a prudent choice, preferring partners of lower quality because it implies limited risk of costly interferences with better competitors (Fawcett & Johnstone 2003, Härdling & Kokko 2005, Venner et al. 2010). Mating preferences can also change with age. In the garter snake *Thamnophis sirtalis parietalis* for example, an ontogenetic shift of mating preference has been described in males (Shine et al. 2001). Large, older males tend to prefer to court larger females while smaller younger males court smaller females, hence presumably leading to size assortment within pairs.

In gammarids, some physiological constraints have been suggested to affect male mating preferences. While moulting, males are not able to hold their current female in precopula any more due to the softening of their cuticle and, with it, the softening of their dactyli that allow female's grasping (Ward 1984b, Franke 1993). As a consequence, we would expect males to choose to pair with females which are closer to moult than they are. If

they moult before their female do, they have to release her before she becomes available for copulation. Such a state dependent decision rule (male's threshold of female's acceptance varies with male's own time left to moult) may be of interest to explain size-assortative mating because individual moulting cycle length directly increases with individuals' body size. We therefore investigated the role of this mechanism in generating size-assortative mating (the whole study is reported in manuscript 2). Using an individual based model, we studied the effect of pairing processes over several moulting cycles, individuals getting one day closer to their moult at each time step of the simulation. The full R code for the model can be found in appendix 3. Pair formation was solely under the control of the state dependent decision rule used by males. Males were also able to perfectly assess females' maturity and to pair accordingly with females closer to moult than themselves (we later relaxed this assumption, see manuscript 2). After several moulting cycles, we looked at the pattern of size assortment within pairs. We found size-assortative mating that varied according to sex-ratio, with strong homogamy for male-biased sex-ratio and weaker, almost inexistent homogamy for female biased sex-ratio. This is the first attempt to explain size-assortative mating in mate guarding crustaceans from a male decision rule that is not based on female body size. This also emphasizes the fact that mating patterns cannot be directly inferred from mating preferences and that mating patterns do not necessarily inform about the underlying pairing process (Burley 1983).

We called this new possible cause for homogamy the female-sooner norm hypothesis in reference to the male-taller norm well-described among human mating strategies (Gillis & Avis 1980, Courtiol et al. 2010). In humans, women tend to pair with men exclusively taller than they are (Gillis & Avis 1980). A parallel can be made between this state dependent preference in women and the tendency of crustacean males to pair exclusively with females closer to moult than they are.

2.1 Indirect evidences for the female-sooner norm

Although it is still not clear whether males actually pair up according to the female-sooner norm, some empirical evidences in mate guarding crustaceans indirectly suggest that they are. In box 1, I presented some predictions about the number of females that should be accepted by males using the female-sooner norm in experimental situation. In addition, this section reviews a few studies reporting males' behaviours that could be related to the female-sooner norm. One of the most compelling clues comes from a study of Bollache and Cézilly

(2004a) which showed that in *G. pulex*, males pairing propensity depended on their time left to moult, as males closer to moult were less inclined to pair up than males further from moult. In addition, they reported that males found unpaired in the field were significantly closer to moult than males in precopula pairs. This latter result has also been described in another species of amphipod, *Gammarus aequicauda* (Thomas et al. 1998). If males use the female-sooner norm, when pairs separate at the time of female's moult, males are rather close to moulting. The female-sooner norm is therefore a possible hypothesis to explain these findings. Lemaître et al. (2009) also showed that among males which had recently copulated after a period of precopula, only 42% paired again with a new randomly assigned female. In addition, accepted females were significantly closer to moult than rejected females. Similar to predictions made when males use the female-sooner norm, this result suggests that males close to moult after a long lasting precopula tend to avoid pairing with females far from moult. Another indirect evidence for such a decision rule has been observed in an isopod, *Idotea baltica*, where males close to moult waited until their next moult to pair again (Borowsky 1987). Note that these empirical observations are not proofs for the occurrence of the female-sooner norm in mate guarding crustaceans. However, we suggest that future studies should acknowledge such male decision rule when studying pairing processes.

Box. 1: proportion of accepted females when males use the female-sooner norm

The large majority of experimental procedures to test mate choice in mate guarding crustaceans involve individual sampled already paired in the field. This is to make sure that individuals are sexually mature and therefore able to pair again in the lab. Although male mate choice has especially been tested in situations of simultaneous encounter of several mates, a few studies have considered sequential encounter of potential mates. For this latter type of experiment, one unpaired male is housed in a cup with one randomly chosen unpaired female. The number of mate rejection is then measured across several trials.

In this box, I will present the results of a small computer simulation mimicking this experimental design. The R code for the simulation can be found in Appendix 4. Let us consider a population of unpaired individuals with an equal number of males and females. Individual body sizes follow a normal distribution of mean $\mu_m = 2.75$ for males and $\mu_m = 2$ for females and of standard deviation $\sigma = 0.2$. The length of their moulting cycle M_{max} directly

correlates to their body size with larger individuals having greater M_{max} ($M_{max} = 14.83 \times$ body size + 6.75, e.g. in *G. pulex*, L. Bollache unpublished data). However, they can be at any time within their moulting cycle, so that their time left to moult M is randomly chosen between 0 and M_{max}. We considered that males paired according to the female-sooner norm, rejecting females closer to moult than they are. We first randomly assigned a hundred males to a hundred females in order to simulate pairing occurring in the field prior individual collection for experiments. In the field, we predicted that the proportion of females accepted by males should be 0.62, C.I [0.52; 0.71]. Now, consider that we only take these 62 pairs previously formed in the field for subsequent experiments in the lab. Similar to classical experiments, after separating partners, we randomly assign males and females and measured the proportion of couple formed. We predicted that males should accept females at a proportion of 0.86 C.I [0.78; 0.93].

In a similar experiment, Dick and Elwood (1989) housed 50 dyads involving one male and one female (both found previously paired in the field with other partners) in separate glass cups. After 20 minutes, they observed 42 precopula pairs. This corresponds to 84% of pairing. In the 8 remaining cups, males have presumably rejected the female. When bootstrapping this result one thousand times, this gives a proportion of accepted females of 0.84 C.I [0.74; 0.94]. This is highly consistent with our predicted value of 0.86. In the experiment that we presented in manuscript 1, males paired with 112 females out of 122. This represents a proportion of accepted females of 0.92 C.I [0.86; 0.96], also highly consistent with our prediction.

This result does not directly prove that males use the female-sooner norm in mate guarding crustaceans. To prove it, we would have to observe pairing processes under natural conditions. However, this represents an example of its potential to explain observed mating patterns.

3. Cryptic diversity and size-assortative pairing

In previous sections, I reviewed different mechanisms that have been empirically and theoretically tested in order to explain the particular pattern of size-assortative pairing. On the contrary, researchers sometimes use observations of size-assortative mating in the field to infer particular mechanisms of pair formation. This approach should be particularly subject to caution because several mechanisms can lead to a particular pattern (i.e. the concept of *equifiniality*, Burley 1983). It is tempting from meta-analysis of the strength of size-assortative pairing between different populations or species to infer general properties that may create this mating pattern (e.g. Arnqvist et al. 1996). However, some unexpected biases potentially associated with pairing processes within each population may prevent such generalisation. In manuscript 3, we showed that cryptic diversity occurring within gammarids lead to miscalculations of size-assortative pairing. A previous study has reported the existence of cryptic diversity in two species of amphipods present in the rivers of Burgundy (Lagrue et al. in prep). Many sexually isolated groups of gammarids have been found living in sympatry. In addition, the mean size of individuals between these groups often differed. Without molecular characterization of each sampled individual, it is difficult to distinguish between individuals from different groups in sympatry which may lead to biases when measuring size assortment among couples. Similar to the habitat heterogeneity presented above, even under random assortment within each group, the body size difference of individuals between groups would possibly lead to the measure of overall size assortment within couples. We tested for such spurious correlations in the 10 rivers we sampled and where two groups of non-interbreeding gammarids were found in sympatry. We measured specific size-assortative pairing within each group. We also measured overall size-assortative pairing within rivers taking into account individuals from both groups for the analysis. Although this revealed positive size assortment among pairs within groups, overall size-assortative pairing was often greater than specific one found within groups. Size-assortative pairing could therefore be overestimated in natural gammarid populations. This is of great importance when interpretations are made from the pattern of size-assortative pairing. For example, we observed strong variation in the strength of overall homogamy between rivers. A common interpretation would conclude for a possible effect of river specific characteristics on size-assortative pairing. If we acknowledge the cryptic diversity occurring within these rivers, we

would instead conclude for a possible effect of mean body size between individuals of different groups on the pattern of size-assortative pairing.

4. Conclusion

Models are, by definition, not reality. However, they are informative when studying the plausibility that particular mechanisms create a given pattern. The female-sooner norm hypothesis is a plausible explanation, among many others for the occurrence of size-assortative mating in mate guarding crustaceans. Experimental insights about pairing processes in mate guarding crustaceans are not all in accordance with our hypothesis. For example, laboratory experiments involving several unpaired males and females housed in a tank have shown that size-assortative pairing can arise within a few hours, suggesting that it results from rapid pairing processes (Bollache 2001, L. Bollache personal communications). Our model, on the other hand, creates size-assortative mating after a few moulting cycles when starting from randomly picked individuals. We do not aim to replace a dogma by another. Rather, we think that it is imperative to acknowledge the importance of considering the whole pairing process when studying the link between mechanisms and potentially resulting patterns in order to avoid inferential fallacies (Burley 1983). Besides, we believe that male mating preferences and male competitiveness should be studied with more scrutiny under realistic situations of competition for females in order to understand their role in the establishment of particular patterns.

Manuscript 2

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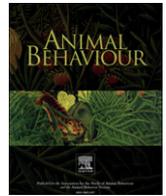
Assortative mating by size without a size-based preference: the female-sooner norm as a mate-guarding criterion

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The study of size-assortative mating, or homogamy, is of great importance in speciation and sexual selection. However, the proximate mechanisms that lead to such patterns are poorly understood. Homogamy is often thought to come from a directional preference for larger mates. However, many constraints affect mating preferences and understanding the causes of size assortment requires a precise evaluation of the pair formation mechanism. Mate-guarding crustaceans are a model group for the study of homogamy. Males guard females until moult and reproduction. They are also unable to hold a female during their own moult and tend to pair with females closer to moulting than them. Using a theoretical approach, we tested the potential for size-assortative mating to arise from such a state-dependent male decision rule. Consistent with previous experimental observations, we found a pattern of size assortment that strengthened with male–male competition over females. This decision rule, which we call the female-sooner norm, may be a major cause of homogamy in mate-guarding crustaceans. This highlights the potential for size assortment to arise from preferences not based on body size and emphasizes the importance of considering pair formation processes when studying the link between preference and mating pattern.

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Mating partners are often found to resemble each other in various traits, such as colour, age or body size (Ridley 1983). This pattern, called positive assortative mating or homogamy, is particularly widespread in nature. Size-assortative mating, defined as a correlation between male and female size among couples in a population, has been well described in several taxa, including birds (Helfenstein et al. 2004), reptiles (Shine et al. 2001, 2003), fishes (Baldauf et al. 2009) and humans (Courtiol et al. 2010). Most notably, it is a very common mating pattern in insects (Arnqvist et al. 1996) and crustaceans (Bollache & Cézilly 2004a).

Because it restricts gene flow within populations, homogamy can have major effects on sexual selection and speciation and is the subject of intense research (Kirkpatrick 2000; de Cara et al. 2008). Beyond its evolutionary consequences, the causes of homogamy remain largely unknown. However, the link between the behavioural traits and the resulting mating pattern is rarely straightforward. For a full understanding of the evolution of these traits, we need to consider not only the consequences of a particular mating pattern on gene flow but also the underlying mechanisms by which they lead to such a pattern. That is why the mechanisms leading to

size-assortative mating have been a major research topic over the past three decades (Parker 1983; Ridley 1983; Venner et al. 2010). Crespi (1989) proposed that size-assortative mating results from three nonexclusive mechanisms. First, physical constraints can prevent mismatched pairs from achieving mating. For example, a male could be physically unable to pair with a female too large or too small compared with his own size, therefore making mismatched pairs less frequent than size-assorted pairs (e.g. Han et al. 2010). Second, if same-sized mates co-occur in time or space, mating should be size-assortative. Individuals of different sizes sometimes have different periods of receptivity for pairing (Miyashita 1994) or have been found in different habitats (Bollache et al. 2000). Third, size-assortative mating can be observed in a population in which one or both sexes are exerting directional preference towards larger mates (Johnstone 1997). When each male prefers large mates, size-assortative mating arises if larger males also outcompete smaller males for access to preferred females, leaving them to pair with smaller females (e.g. Fawcett & Johnstone 2003; Härdling & Kokko 2005; Venner et al. 2010). When females also prefer larger males, smaller individuals of both sexes are rejected by larger mates and size-assortative mating occurs (Parker 1983). Directional mate preference for large partners has been extensively explored since Crespi (1989). It remains, by far, the most commonly invoked process to explain size-assortative mating in nature (e.g. Elwood et al. 1987; Brown 1990; Rowe &

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Arnqvist 1996; Beeching & Hopp 1999; Shine et al. 2001; Baldauf et al. 2009; but see Taborsky et al. 2009).

However, studying the link between a mating preference and a mating pattern is highly challenging (Wagner 1998; Widemo & Sæther 1999). A mating pattern results from the interaction between individuals' preferences and internal or external constraints that may act on these preferences (Cotton et al. 2006). For instance, scramble competition in mating (i.e. competition in which individuals' access to mates is solely constrained by the pairing success of competing individuals) is likely to constrain strongly the availability of potential partners, therefore limiting access to preferred mates. In that context, observations of individual preferences in the absence of competition, as reported in several experimental studies, do not necessarily account for a particular mating pattern (Wagner 1998). Reciprocally, an observed pattern of size-assortative mating is not sufficient to identify the traits targeted by the underlying preference, nor it is enough to infer either the shape of the preference function or the decision rule used to discriminate mates. Individuals may base their preferences on a variety of traits other than body size that reflect the quality of their potential partners. Also, apart from directional preference for larger mates, preference functions may sometimes depend on an individual's own quality (Alpern & Reyniers 1999). They could either prefer to mate with like (i.e. homotypic preference; Burley 1983; Cézilly 2004) or discriminate between potential mates according to a state-dependent threshold (Riebel et al. 2010). Homotypic or state-dependent preferences have rarely been invoked to explain assortment by size (but see Kalick & Hamilton 1986), nor have been mating preferences based on traits other than size.

Size-assortative mating is usually reported when pairs are conspicuous and easily identified. This is the case in species in which mating partners share parental investment or display pre- or postcopulatory mate guarding. It is probably why size-assortative mating in mate-guarding crustaceans has been the subject of an extensive literature (e.g. Birkhead & Clarkson 1980; Adams & Greenwood 1983; Elwood et al. 1987; Iribarne et al. 1996; Bollache & Cézilly 2004a, b; Franceschi et al. 2010), although its proximate mechanisms are still poorly understood (Sutherland et al. 2007). In mate-guarding crustaceans, individuals grow continuously throughout their lives after each moult. An individual's intermoult duration (the time between two successive moults) increases with body size. Females are only receptive for copulation for a short period of time, as their eggs can be fertilized for only a few hours after their moult. The strong male–male competition for access to receptive females favoured the evolution of long-lasting precopulatory mate guarding, as guarding a female earlier in her intermoult period provides the male with a competitive advantage (Parker 1974; Grafen & Ridley 1983; Jormalainen 1998). Perhaps owing to this close link between precopulatory mate guarding and sexual selection, size assortment in this mating system has often been considered to result from a directional male mating preference for larger, more fecund females combined with a size bias in male competitive ability (e.g. Elwood et al. 1987; Elwood & Dick 1990; Bollache & Cézilly 2004a; Sutherland et al. 2007). Larger males are commonly expected to have a competitive advantage over smaller ones in gaining access to a preferred female. They can usurp larger females from other males after take-overs (Ward 1983) or invest more energy in mate guarding than smaller males (Elwood & Dick 1990).

Surprisingly, other areas of amphipod biology have been overlooked in explanations of size assortment. Males have been described as unable to guard a female during their own moult (Ward 1984). Because mating is only ensured if a male holds a female at the time of her moult (i.e. female sexual receptivity), males should decide to pair with females that moult before they do (Thomas et al. 1998; Bollache & Cézilly 2004b). Although mating preference based on time left to moult as been studied in amphipods (Birkhead & Clarkson 1980; Ward 1984; Elwood et al. 1987; Galipaud et al. 2011), its potential role in leading to size assortment has almost never been investigated.

In this study, we tested the overlooked hypothesis that a state-dependent decision rule based on time left to moult is sufficient to lead to size-assorted pairs. Using an individual-based model, we studied pair formation when males decided to pair with females that moulted before themselves and we observed the resulting mating pattern. Unlike other hypotheses, we did not consider any interference between males or any effect of female behaviour. However, we explicitly took scramble competition into account and we never assumed any preference function or decision rule based on body size.

THE MODEL

We parameterized the model in reference to the biology of *Gammarus pulex*, a well-studied species of amphipod crustacean, but we kept it as general as possible in order to fit the biology of most species of crustaceans with continuous growth. All individuals were sexually mature. Each individual was defined by its sex, mating status (unpaired or paired) and its size, S (usually measured in millimetres in *G. pulex*). Male and female sizes were drawn from normal distributions with means μ_m and μ_f, respectively, and standard deviation σ. By default, we used $\mu_m = 2.75$ mm and $\mu_f = 2$ mm, as these are the mean sizes of the fourth coxal plate (used as a proxy of body size) measured in natural populations of *G. pulex* (Bollache & Cézilly 2004a). Sexual size dimorphism (SSD) was represented as the ratio μ_m/μ_f. As default value, we chose SSD = 1.375, which roughly corresponds to the SSD found in natural populations of *G. pulex*. The length of an individual's moulting cycle M_{max} (in days) was assumed to increase linearly with its body size ($M_{max} = 14.83 \times S + 6.75$; Fig. 1; e.g. in *G. pulex*, Galipaud et al. 2011; L. Bollache, unpublished data). The time left to the next moult, M (in days; Fig. 1), equalled M_{max} immediately after a moult, but declined by one unit each day in between moults. After each moult, individuals grew in size by a factor g, the relative growth rate (by default $g = 1.1$). When a paired female moulted, she became receptive for copulation, after which the couple separated. When a paired male moulted, he could not hold his female anymore, so the couple separated (Ward 1984). Every day, each individual had a probability d of dying ($d = 0.012$ by default). Individuals thus had a life expectancy of 83.3 days and 99% of them died before reaching 380 days. This is consistent with the life span observed in natural populations of *G. pulex* (Sutcliffe 1993). Every **Q1** dead individual was replaced by a mature individual of the same sex and of a size chosen from the normal distributions described above. This ensured that population size and sex ratio were constant. If an individual died while paired, its partner immediately became available for re-pairing.

The population was composed of N individuals of both sexes. The numbers of males and females depended on the sex ratio SR, defined as the proportion of males. To simulate reproductive asynchrony, individuals entered the population with a value of M chosen randomly from the distribution of all possible values between 0 and M_{max} (Fig. 1). Pairings occurred through male mate choice only. Males only paired with females that would moult sooner than themselves, thereby preventing premature pair separation caused by their own moult (this assumption is relaxed in latter analysis, leaving the possibility for males to make errors). Each time step t of the simulation represented 1 day for individuals. At each t, we ordered the unpaired males randomly and then gave
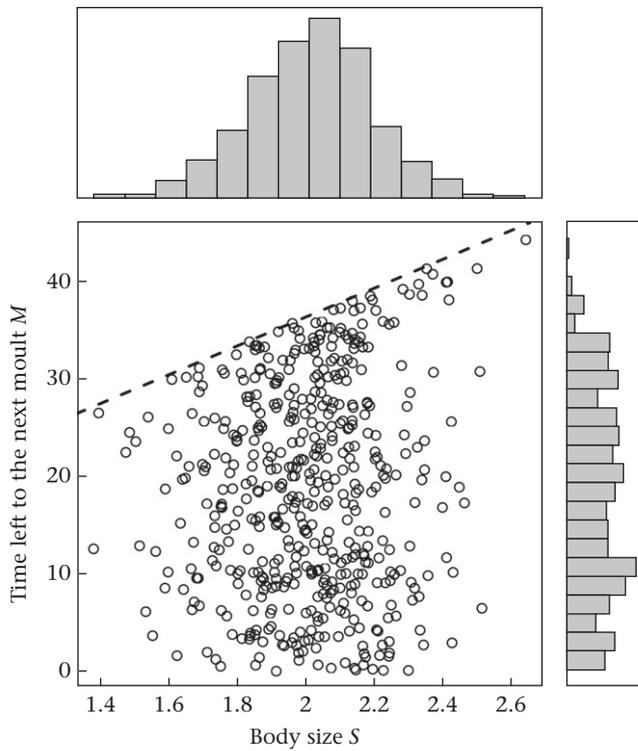
Figure 1. Time left to the next moult as a function of body size. The dashed line represents the correlation between body size and maximum time left to moult. As moulting was not synchronous, at the beginning of the simulation individuals ($N = 1000$) entered the population with a time left to moult randomly chosen among values between 0 and M_{max}. This resulted in a distribution of M almost uniform in a population, although individual body size S followed a normal distribution.

each one in turn the opportunity to pair. For a given male, a mate was randomly chosen from the remaining unpaired females that met his guarding criterion ($M_f < M_m$), if any. After being assigned to a particular male, a female was not available for pairing with other males before she was released by her current partner. Pairs remained together until the female moulted or one of the two partners died. After separation, males and females were immediately available for pairing with a new mate. The model was written in R language (R Development Core Team 2012).

Simulations

We allowed these cycles of guarding and mating to continue until the pattern of size-assortative mating had reached an equilibrium, which always happened within 1000 time steps (t_{max}). We assessed the degree of size assortment between mating partners with the Pearson coefficient of the correlation between male and female size in pairs (Arnqvist et al. 1996). Pairing sequence may be subject to variations between replicates of a given simulation. To make sure that we could draw conclusions from the observed pattern, we ran *r* replicates of the same simulation (i.e. with exactly the same set of parameter values) and considered the mean response for interpretation. Because the availability of partners influences mating patterns, we first assessed the effect of the sex ratio SR on size-assortative mating. Second, we considered the effects of *g*, *d* and SSD on homogamy for size.

At t_{max}, we also looked at the size of unpaired and paired individuals within each sex. To guarantee independence between observations, we randomly sampled one individual at t_{max} for each repetition of the simulation among unpaired (for 500 repetitions of the simulation) and paired individuals (for a separate set of 500

repetitions). We then assessed the strength of the disparity in size between unpaired and paired individuals, calculating the Cliff's δ as a measure of effect size (Nakagawa & Cuthill 2007). Following the same procedure, we measured the time that paired males spent in precopula with a particular female by looking at the M_f of their current partner at t_{max}. We also tested for an effect of male body size on precopula duration with a linear regression model.

Individual's Error in Choice

In nature, males are unlikely to be able to assess perfectly a female's time left to moult relative to their own before engaging in precopula. We therefore added errors in male's decision making in our simulations (McNamara et al. 1997). When encountering a female, a male had a probability *P* of accepting her, given by:

$$P = \frac{1}{1 + e^{-\lambda(M_m - M_f)}}$$

,where M_m and M_f represent the male and the female time left to moult, respectively, and λ controls the accuracy of male choice. The greater the value of λ, the better the male can assess the female's time left to moult. When $M_m >> M_f$, $P \approx 1$ whereas when $M_m << M_f$, $P \approx 0$.

RESULTS

All simulations led to positive size-assortative mating. The strength of homogamy varied according to the sex ratio (Fig. 2). One important mechanism in creating the mating pattern was a disadvantage of small males with relatively short M_m in getting access to large females with long M_f. However, this mechanism only led to size assortment under particular conditions of male–male scramble competition for pairing.

There were two ways for pairs to split up. Separations were either caused by the death of one of the two partners or, in the vast majority of cases, by the female's moult. Under low SR, females were abundant in the population, male–male scramble competition was low and size-assortative mating was weak (Fig. 2). Newly released females did not always immediately find a new male with
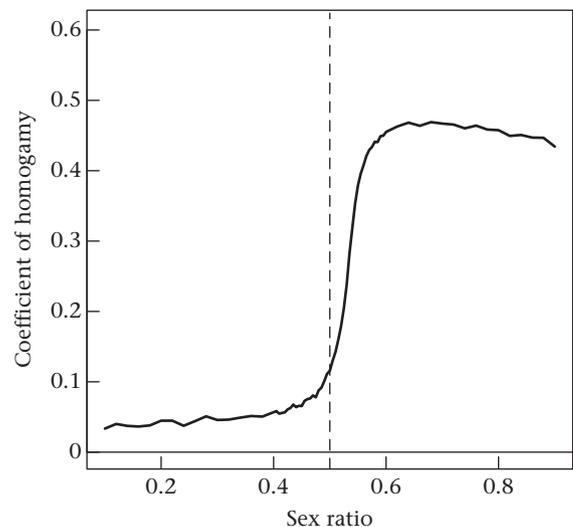
Figure 2. Coefficient of homogamy (measured as the mean Pearson coefficient of the correlation between male and female body size in pairs over 500 simulations) as a function of sex ratio. The vertical dashed line indicates an even sex ratio (0.5). Parameters: $N = 1000$, $d = 0.012$, $g = 1.1$, SSD = 1.375.

which to form precopula. To do so, they had to wait for a few days, bringing them closer to the moult. Because both large and small females sometimes did not pair until close to their moult, this resulted in a weak correlation between female size and M_f. Unpaired males were thus likely to pair with females of any size whatever their own M_m. That is why we observed only weak size-assortative mating for low values of SR (Fig. 2). When SR reached higher values, male–male scramble competition increased and size-assortative mating was stronger (Fig. 2). Each male that secured a female strongly affected the pairing success of other males. After their moult, females rapidly entered into precopula with a new male. Newly released males were close to their moult and were therefore unable to find a female meeting their guarding criterion. In order to pair with a new female, they had to wait until their own moult and the beginning of a new moulting cycle. Size and M_m were therefore correlated in males that were able to pair. In a nutshell, with increasing male–male competition, there was a strong correlation between size and time left to moult in the population of unpaired individuals that were able to pair. Under these circumstances, small males had a disadvantage in access to large females with $M_f > M_m$, which resulted in more frequent assorted pairs (Fig. 2). This also explains why large females were less likely than smaller females to be found in precopula and why unpaired males were smaller than paired males (Table 1). In addition, large males tended to be passively trapped for a long time with females, therefore spending more time in precopula than smaller males (SR = 0.4: $t_{198} = 1.29$, $P = 0.2$, slope = 1.75, CI from −0.91 to 4.41; SR = 0.5: $t_{198} = 1.02$, $P = 0.3$, slope = 1.24, CI from −1.14 to 3.63; SR = 0.6: $t_{198} = 6.13$, $P < 0.0001$, slope = 7.93, CI from 5.40 to 10.47). Thus, at any time, large males were more likely to be paired than small males. This also accounts for the size difference between paired and unpaired males we observed (Table 1) and is consistent with previous experimental studies (Rowe & Arnqvist 1996). By segregating individuals according to their size, this passive accumulation of large males with large females resulted in even stronger size-assortative mating. However, passive accumulation alone is not sufficient to explain size assortment. Without a state-dependent decision rule, size-assortative mating was no longer found. To sum up, when male–male scramble competition increased, this strengthened the correlation between size and time left to moult among males and females that formed precopula. Small males were unable to pair with large females far from moult, therefore creating size-assortative mating at a population level. The necessary component for homogamy to arise was the positive correlation between S and M. Without this correlation, no size-assortative mating was observed.

It is worth pointing out that at SR = 0.5, some newly released unpaired males were close to moulting and were unable to find a mate meeting their guarding criterion, owing to the long M_f of unpaired females (i.e. females that just began a new moulting cycle). Despite there being an equal number of males and females in the population as a whole, the actual number of unpaired males able to pair (i.e. with a large M_m) was still lower than the number of

available unpaired females. The operational sex ratio (OSR; here defined as the relative number of males and females available for pairing, not for mating; Lemaître et al. 2009) was thus female-biased and the strength of male–male competition was still low. This accounts for the relatively low size-assortative mating we observed at SR = 0.5, before it rapidly increased as the OSR became biased towards males (Fig. 2).

Size assortment was also sensitive to individual relative growth rates and the probability of dying. For these parameters, the default values we chose led to a weaker pattern of size assortment than expected under slightly different conditions. Mates were more strongly assorted by size when they were less susceptible to individual mortality (Fig. 3a) or when they grew more at each moult (Fig. 3b). Size-assortative mating also increased when males and females tended to be similar in size (Fig. 4). Under low SSD, males and females tended to be more similar in their M_{max}. Several females had their M_{max} greater than small males' M_{max}. The size bias in pairing success among males was therefore strengthened because small males were even less likely to encounter a large female meeting their guarding criterion. Size-assortative mating was resistant to errors in male assessment of female time left to moult (Fig. 5).

Table 1
Size disparity between unpaired and paired individuals within each sex

Sex ratio	Male		Female	
	Cliff's™	95% Confidence interval	Cliff's™	95% Confidence interval
0.4	−0.15	−0.21 to −0.07	0.06	−0.01 to 0.14
0.5	−0.08	−0.15 to −0.01	0.19	0.12 to 0.26
0.6	−0.19	−0.26 to −0.12	0.99	0.96 to 1.00

Negative values of Cliff's™ indicated that paired individuals were larger than unpaired individuals while positive values indicated the opposite.


Figure 3. Effect of (a) probability of dying d and (b) relative growth rate g on size-assortative mating, for three values of the sex ratio (SR = 0.4, SR = 0.5 and SR = 0.6). In both graphs, dashed lines indicate the default values of (a) d and (b) g. Parameters: $N = 1000$, SSD = 1.375.
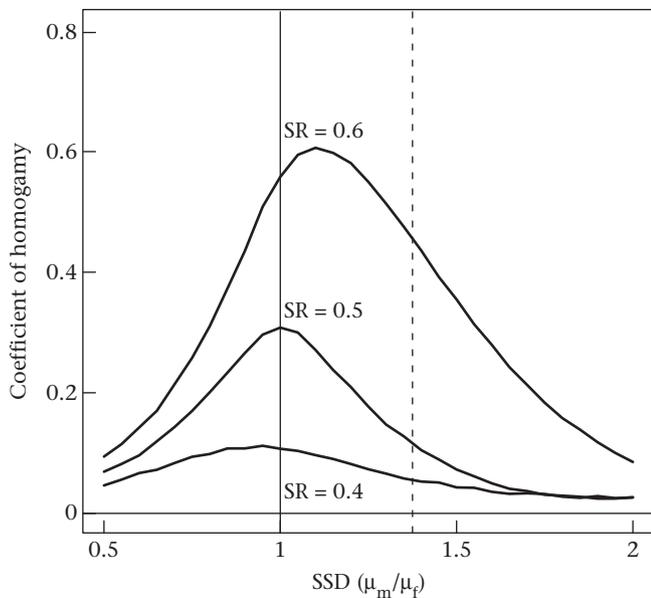
Figure 4. Effect of sexual size dimorphism on size-assortative mating for three values of the sex ratio. At SSD = 0 (solid line) the male and female size distributions are identical ($\mu_m = \mu_f = 2$; $\sigma = 0.2$). The dotted line indicates the default value of SSD. Parameters: $N = 1000$, $d = 0.012$, $g = 1.1$.

DISCUSSION

We showed that it is possible to find size-assortative mating without assuming either a preference function or a decision rule based on body size. This contrasts with previous work on amphipods, which considered male mating preference for larger, more fecund females as the main mechanism leading to homogamy (Elwood et al. 1987; Bollache & Cézilly 2004a; Sutherland et al. 2007). One could argue that our result is only a by-product of the weak correlation between size and time left to moult we found in the simulations. A preference based on time left to moult would then actually be a preference for body size. If so, males would presumably prefer, and most likely pair with, larger, more fecund females that also happen to be far from moult. This is precisely the opposite of the pattern reached in the model, with males tending to pair with smaller females rather close to moult, leaving larger

females unpaired (e.g. Hatcher & Dunn 1997). The mate-guarding criterion we modelled based on time left to moult did not act as a directional mating preference for large females.

The state-dependent male decision rule we assumed is comparable to the male-taller norm in human mating (Gillis & Avis 1980). Human females are described as preferring to consort with males that are exclusively taller than them. This human mating strategy has also been shown to lead to size-assortative mating (Courtiol et al. 2010). Similarly, in our model, we considered that males would tend to pair exclusively with females closer to moult than they are. This female-sooner norm represents a novel hypothesis to explain size-assortative mating in crustaceans.

The effect of variation in mate-guarding duration has previously been invoked to explain size assortment. Some authors have argued that in reproductive systems where larger individuals have longer-lasting breeding periods, larger males would tend to accumulate passively with larger females, hence leading to size assortment (McCauley & Wade 1978). However, according to our results, this 'passive accumulation' alone is not a sufficient mechanism to explain the pattern of size assortment. Another previous hypothesis, called the 'timing hypothesis' (Elwood & Dick 1990) also suggested that, because males incur an energy cost in precopula, there should be a size bias towards males' ability to guard females. Large males, with more energy, should be more successful in guarding females over a long period of time compared with smaller males. According to this hypothesis, every male prefers larger females also further from moult than smaller females. Large males are better able to overcome the costs of guarding them, hence leading to size assortment. Our hypothesis differs on two points from the 'timing hypothesis'. First, the female-sooner norm we proposed does not consider costly precopulas for males. Even without consideration of size or energetic reserves, males close to moult were less likely to find a female meeting their guarding criterion. Under strong male–male competition, small males tended to be closer to moult than large males. This resulted in a size bias in male access to unpaired females and eventually to size-assortative mating. Second, the 'timing hypothesis' predicts that males should trade female size against time left to moult to choose partners in order to maximize the number of offspring they sire per guarding event (Elwood et al. 1987). In our study, males based their choice solely on female time left to moult, which led to size-assortative mating even under rather strong errors in assessment. In that
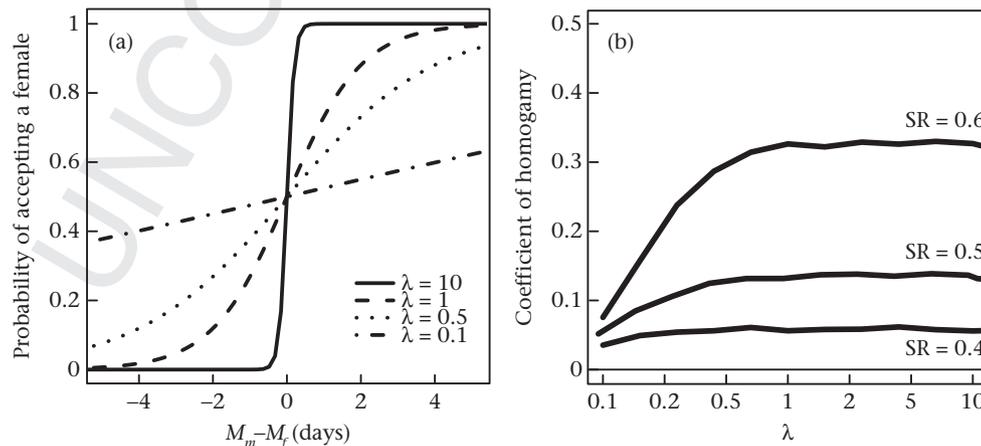
Figure 5. Male errors in mate choice. (a) When males are capable of perfect assessment of female M_f relative to their own M_m ($\lambda \geq 10$), male mate choice occurs without mistakes and males pair with females only if the difference $M_m - M_f$ is positive. When λ decreases, the male probability of making an error and accepting a female further from moult than himself increases. The effect of λ on the coefficient of homogamy is represented in (b) for three values of the sex ratio (SR = 0.4, SR = 0.5 and SR = 0.6). Parameters: $N = 1000$, $d = 0.012$, $g = 1.1$, SSD = 1.375.

sense, we suggest a parsimonious alternative to explain homogamy in mate-guarding crustaceans.

In our model, male–male scramble competition for access to females is the main mechanism to explain size-assortative mating. Size-assortative mating strongly increased with a more male-biased sex ratio. This is highly consistent with previous observations of homogamy in crustaceans (Bollache et al. 2000; Bollache & Cézilly 2004a). We also found that paired males tended to be larger than unpaired males. This has also been observed in previous studies (Birkhead & Clarkson 1980; Ward 1986). Yet, authors often erroneously interpret this pattern as evidence for large males having priority of access to larger, preferred females. Here we have shown that it is possible to obtain these patterns without any size bias in male capacity to undergo precopula or to dislodge competitors from preferred females (e.g. take-over). Because mating patterns potentially arise from several processes, mating preferences or biases in mating success cannot be inferred only from patterns of size-assortative mating at the population level (Rowe & Arnqvist 1996).

It is also worth noting that the very same decision rule can lead to wide variation in the level of homogamy according to environmental conditions. Death rates, relative growth rates and sexual size dimorphism all affected the strength of size assortment in our model. Populations under different conditions of predation, food availability or selection pressure on growth may therefore vary widely regarding the strength of size assortment between mating partners, even if the main mechanism it results from remains the same. This could partly account for the strong intraspecific variation in size-assortative mating observed between different natural populations (e.g. Ward 1986; Arnqvist et al. 1996; Bollache et al. 2000; Bollache, unpublished data).

Conclusion

The female-sooner norm represents a novel hypothesis to explain size-assortative mating. It is embedded in the biology of mate-guarding crustaceans, a particularly well-studied group when it comes to homogamy. Unlike previous hypotheses, this one considers size assortment to result from a decision rule not based on body size. Males tended to pair with females that moulted sooner than themselves. State-dependent preferences are not as restrictive as a directional preference, so males still found plenty of potential mates meeting their criterion. Competition among males is also presumably relaxed under such a preference, making its maintenance easier to explain within natural populations (Barry & Kokko 2010). This highlights the fact that there is not necessarily a direct relationship between preferences and mating patterns. There are likely to be many constraints on pairing processes resulting from a particular mating preference under natural conditions. Its observation under controlled environments using specific experimental procedures is not sufficient to infer a mating pattern at the level of the population. There is a need for a better understanding of pairing processes leading to mating patterns in order to link preference functions and decision rules to actual reproduction, and thus evolution.

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Manuscript 3

Galipaud, M., Bollache, L., Wattier, R., Dechaume-Moncharmont, F-X. and Lagrue, C. Overestimation of the strength of size-assortative pairing in taxa with cryptic diversity: a case of Simpson's paradox. *In prep*

Overestimation of the strength of size-assortative pairing in taxa with cryptic diversity: a case of Simpson's paradox.

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Abstract:

Size-assortative pairing is one of the most common pairing patterns observed in nature. It is widespread in crustaceans, taxa from which cryptic diversity is regularly reported. Consequently, previous measures of size-assortative pairing in crustacean species may have been biased by the occurrence of previously undetected non-interbreeding groups of individuals living in sympatry. To quantify this potential bias, we measured size-assortative pairing among pairs of gammarids in populations containing two non-interbreeding groups living in sympatry. We measured overall and within group size-assortative pairing to test for potential effects of cryptic diversity on homogamy. We found positive size-assortative pairing in almost every group. However because of individual body size differences between groups, overall size-assortative mating tended to be stronger. We discuss this case of Simpson's paradox in relation to potential inferential fallacies when studying the cause of pairing patterns.

Introduction:

Size-assortative pairing occurs when pair formation during reproduction is non-random and leads to a positive statistical correlation between the body sizes of mates (Crespi 1989, Cézilly 2004). It is one of the most widespread pairing patterns observed in nature and has been described in numerous taxa including birds (Helfenstein et al. 2004), reptiles (Shine et al. 2001), fishes (Beeching & Hopp 1999), mammals (including humans, Courtiol et al. 2010) and most notably insect (Arnqvist et al. 1996) and crustaceans (Ridley 1983). Size-assortative pairing is thought to result from a wide array of mechanisms related to sexual selection and/or conflict (Parker 1983, Crespi 1989, Fawcett & Johnstone 2003, Härdling & Kokko 2005, Venner et al. 2010) but also mechanical (Han et al. 2010), physiological (Myashita 1994, Galipaud et al. *in press*) and environmental constraints (Adams & Greenwood 1983, Bollache et al. 2000). Evolutionary consequences of size-assortative pairing are also extensively studied. When adult body size is fixed, size-assortative pairing, if it leads to mating, can reduce gene flow between size classes thus allowing maintenance of genetic variation within populations and in extreme cases, leading to sympatric speciation (Partridge 1983, Kirkpatrick 2000, Jones et al. 2003). When fecundity increases with body size, size-assortative pairing may also have important consequences on variance in reproductive success among individuals.

Many crustacean species display size-assortative pairing (Ridley 1983, Elwood & Dick 1990, Sutherland et al. 2007, Franceschi et al. 2010). Males hold on to females before copulation, a behaviour called precopulatory mate guarding (also called precopula or amplexus, Jormalainen 1998). Mating pairs are often long lasting, allowing observation of pairing patterns within a given population and making crustaceans ideal models for the study of size-assortative pairing. Cryptic diversity has also been reported in several species of crustaceans (Lefébure et al. 2006, Bickford et al. 2007). Constant and rapid progress in molecular techniques used for species identification increasingly show that morphological identification may under-estimate the number of genetic units within a given taxonomic species. In many freshwater crustaceans, non-interbreeding genetic groups of individuals have been found living in sympatry where a single taxonomic species was previously described (Wellborn & Cothran 2004, Lagrue et al. in prep). The study of pairing patterns in these taxa may have thus been subject to errors. If non-interbreeding groups occur in sympatry, observed pairing patterns may differ from within group patterns. If size-assortative pairing exists within each group, it is possible that no overall size assortment is observed when groups are not

considered for analysis. Conversely, it is possible to generate overall patterns of size assortment among pairs if individual body sizes differ between groups (figure 1a). Simple simulations showed that significant, positive size-assortative pairing can arise in mixed populations even when size differences between groups are small (figure 1b).

This spurious correlation is known as the Simpson's paradox (Yule 1903, Simpson 1951). However, it is rarely considered in an ecological context (but see Allison & Goldberg 2002). In this study, we measured size-assortative pairing in the *Gammarus pulex*/*Gammarus fossarum* species complex. These amphipod crustaceans are difficult to identify morphologically and cryptic taxa have recently been documented to occur in sympatry (Lagrue et al. in prep).

Methods:

A previous study has revealed important cryptic diversity among gammarid populations of Burgundy, France (Lagrue et al. in prep). Authors found non-interbreeding groups of gammarids, referred hereafter as molecular operational taxonomic units (MOTU, Blaxter et al. 2005), living in sympatry in several rivers. The number of MOTUs varied between rivers, and some rivers contained only one MOTU. We collected precopulatory pairs of amphipods using the kick sampling method (Hynes 1954) in 10 rivers that contained only 2 MOTUs (names and GPS localisations of each river can be found in the figure 2 footnote). Genetic identification of each individual was performed using a DNA barcoding method on COI sequences (for details on the molecular identification protocol see Lagrue et al. in prep). We measured gammarids body size using height of the fourth coxal plate as a proxy (Bollache et al. 2004). For each sex within each river, we quantified the difference in individual body size between MOTUs using Cohen's *d* as a measure of effect size (Nakagawa & Cuthill 2007). Within each MOTU, we then quantified the strength of size assortment among precopulatory pairs using Pearson's coefficient of correlation as a measure of effect size (Arnqvist et al. 1996, Nakagawa & Cuthill 2007). For each river, we also measured overall size-assortative pairing considering all individuals in the analysis, regardless of their MOTU. Statistical interpretations on differences between measures of size assortment were made using the 95% confidence interval (C.I.) range around effect sizes. Significant differences between effect sizes were thus assessed by comparisons of their C.I (Cumming & Finch 2005).

Results:

Overall, we collected and genetically identified 1386 gammarids belonging to seven different MOTUs. In five of the ten rivers, some precopulatory pairs were constituted of individuals from different MOTUs (number of mixed pairs: Romaine, 1; Vèze, 2; Ouche, 6; Seine, 5; Résie, 3). However, these mixed pairs represented a maximum of 6% of the overall number of pairs sampled.

Size-assortative pairing within rivers as well as within each MOTU varied in strength but was almost always significant (figure 2). Size-assortative pairing was also fairly consistent within individual MOTU, even across different rivers. Considering 95% CI overlap, the strength of size assortment among pairs did not differ significantly in 4 (3, 5, 6 and 7) out of the 6 MOTUs observed in more than one river. For 2 MOTUs (1 and 2), we found significantly different values of size-assortative pairing between rivers.

Overall values of size-assortative pairing within rivers (i.e. considering MOTUs as a single functional unit in the analysis) varied substantially (Figure 2). Overall values of size assortment among partners were generally stronger compared to values detected in individual, sympatric MOTUs. In 6 rivers, one or both measures of size assortment within MOTU were significantly weaker than the overall measure of size-assortative pairing (Figure 2). This illustrates Simpson's paradox in that the overall measure of correlation between male and female body size overestimated actual size-assortative pairing within MOTUs.

As predicted by theoretical simulations (figure 1b), overall size assortment tended to increase with increasing differences in body size between individuals from two sympatric MOTUs for both males (figure 3a, rho = 0.68, p = 0.035) and females (figure 3b, rho = 0.93, p < 0.001). Unlike the simulation, for which random mating within MOTUs was assumed, field data showed significant, positive size-assortative pairing within most MOTU. This may account for the tendency of observed pattern of overall size assortment to be greater than predicted ones.

Discussion:

Our results show that, according to Simpson's paradox, cryptic diversity may lead to an over-estimation of assortative pairing levels in natural, functional populations. This trend was detected in more than half of the rivers sampled in our study. Measurements of size assortment among pairs made without considering cryptic diversity did not reflect size-

assortative pairing occurring within each MOTU. Such spurious correlation is likely to exist in a number of other taxa where cryptic diversity has been documented, suspected or is likely to occur. In arthropods, especially crustaceans, cryptic diversity is assumed to be common (Witt et al. 2006, Bickford et al. 2007). Since these taxa have also been the subject of most of the studies on size-assortative pairing (Crespi 1989), the reliability of documented measures of the strength size-assortative pairing may need to be re-examined and confirmed in the light of our findings.

This poses several problems when trying to identify causes for observed size-assortative pairing strength. Size assortment among pairs has long been reported to originate from mechanisms of mate choice (Parker 1983, Crespi 1989). For instance, it can occur if individuals prefer to pair with mates of similar phenotype/size, either because assorted pairs have higher reproductive success (i.e. homotypic preference, Burley 1983) or because less competitive individual avoid seeking high quality partners (i.e. prudent choice, Fawcett & Johnstone 2003, Härdling & Kokko 2005). When cryptic diversity occurs, observations of partner rejection based on body size could be mistakenly taken as evidence of prudent choice or homotypic preference. Yet, rejections may actually occur between individuals from non-interbreeding groups differing in mean body size. In that case, Simpson's paradox may lead to misinterpretations of observed mating behaviour and possible errors in our interpretation of size assortment.

Another hypothesis considers size-assortative pairing as a result of spatial distribution of individuals of similar size within populations (i.e. the micro-habitat segregation hypothesis, Birkhead & Clarkson 1980). Even under random assortment of individuals, size-assortative pairing should thus arise in populations due to size-related spatial segregation (Birkhead & Clarkson 1980). This hypothesis has been tested in amphipod crustaceans, individuals of different sizes often occupying different micro-habitats in the river, creating a strong size assortment among pairs in the overall population. It was thus concluded that the observed pairing pattern in amphipods could be induced by size related micro-distribution (Birkhead & Clarson 1980, but see Bollache et al. 2000). Alternatively, it is possible that the different sub-groups found in different micro-habitat and assumed to belong to the reproductively functional unit were actually distinct non-interbreeding MOTUs, as described in this study. If so, size-assortative pairing arose for spurious correlations between male and female sizes of individuals from different MOTUs. Again, the lack of information about cryptic diversity may have led to misinterpretations of the mechanisms causing this pattern.

Confusion can also occur when measuring size-assortative pairing in different populations from several locations. In our study, we observed variations in the strength of overall size-assortative pairing between rivers. Without knowledge of cryptic diversity, measures are assumed to be made among different populations of the same taxonomic unit. Environmental effects may thus appear to be a likely cause for observed variations in size-assortative pairing between rivers. However, in our case, size assortment among pairs increased with increasing size difference between MOTUs within rivers. Variations in the pairing pattern between rivers probably arose from a statistical effect due to variations in mean individual size differences between MOTUs rather than effects related to rivers characteristics. This is also in accordance with the consistency in the strength of size-assortative pairing within individual MOTU across rivers.

Beside these problems, it is worth noting that comparison of size-assortative pairing between sympatric MOTUs may also inform about its causes. For example, in our analysis, the consistent strength of size-assortative pairing within particular MOTUs across rivers argues against environmental causes for variations in this pattern. Instead, within MOTU individuals' specific mating behaviours may account for the strong difference in size assortment among pairs observed between MOTUs. However, such interpretations must be made with caution because inferring causes from observed patterns is subject to limitations (Burley 1983, Galipaud et al. in press).

Conclusion:

The recent discovery of cryptic diversity implies a critical reappraisal of previous findings made in the species involved. Errors in the interpretation of causes and consequences of size-assortative pairing may be present in the literature, due to the genuine ignorance of cryptic diversity in natural populations and the lack of tools to detect such diversity. In most of experimental and field studies, cryptic diversity is, by definition, far from obvious if not actively sought. Errors are therefore likely to continue to occur in fields that are not used to employ phylogenetic. Although sequencing techniques are improving (Gardner et al. 2011), it is still mostly applied to research on population and conservation genetics. Perhaps and maybe hopefully, a time will come when molecular identification will be as common as body size measurements in our labs!

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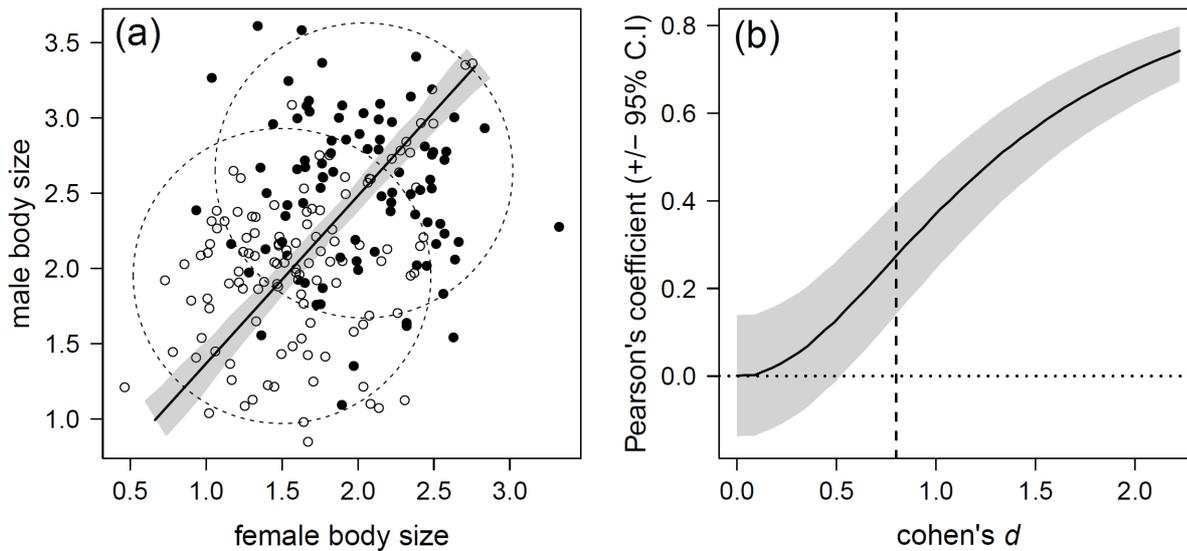
Figure 1. Illustration of Simpson's paradox on size-assortative pairing in taxa with cryptic diversity. [a] plot of male body size against female body size within pairs of two simulated sexually isolated groups (n = 100 pairs each), where males and females mated randomly (i.e. no size-assortative pairing within groups). In both groups, body sizes were drawn from normal distributions. Among individuals of group 1 (white dots), mean body size was μ_{f1}= 1.5 for females and μ_{m1}= 1.95 for males. For individuals of group 2 (black dots), μ_{f2}= 2 and μ_{m2}= 2.65. Within both groups, standard deviation of size distribution was σ = 0.5. The size of the difference between the two groups corresponded to a Cohen's d value of 0.8. Dashed circles represent 95% confidence ellipses for bivariate data. Although no size-assortative pairing occurred within groups, an overall positive size assortment was found when including both group in the analysis (Pearson's correlation coefficient r = 0.31, 95% CI = [0.18; 0.43], p < 0.001). The correlation was illustrated by a robust major axis regression and its 95% confidence interval in grey (Warton et al. 2006): R^2 = 0.096, $P < 0.001$. [b] Simulated effect of body size difference between the two groups aforementioned (measured with a Cohen's d) on the strength of size assortment measured as Pearson's coefficient of correlation (with 95% C.I. in grey). Vertical dashed line corresponded to the situation described in [a].
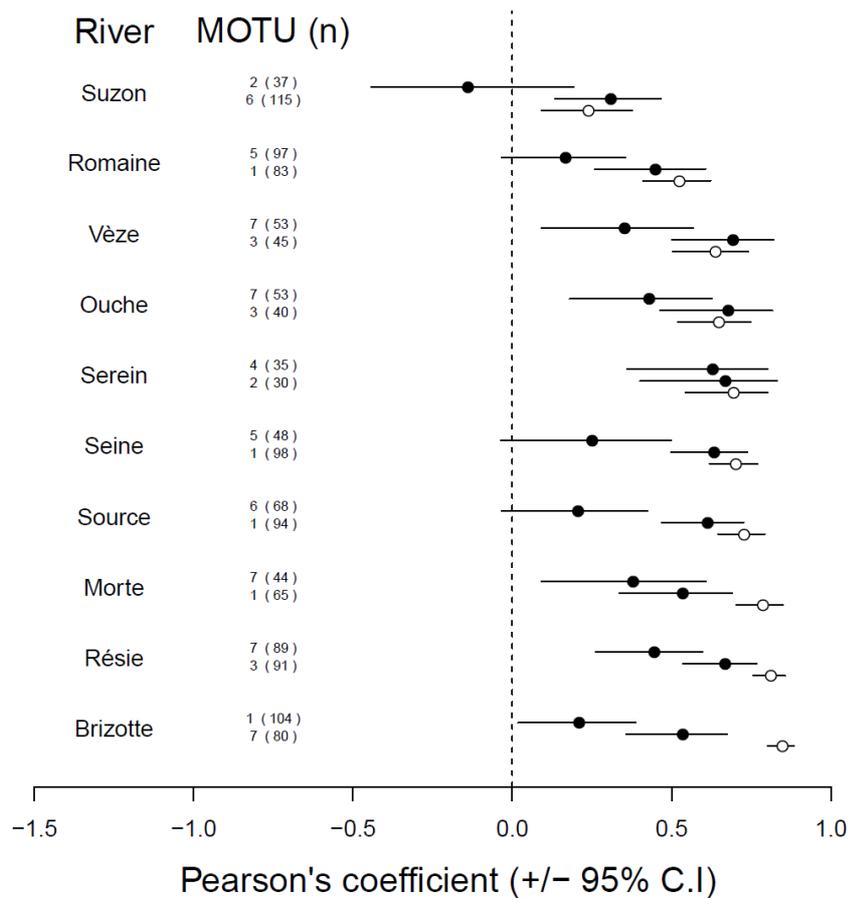
Figure 2. Strength of size-assortative pairing (Pearson's correlation coefficient) within rivers (white dots) and within each MOTU (black dots). Error bars represent 95% confidence interval for each measure of size assortment. Number of sampled precopulatory guarding pairs is given for each MOTU (values in brackets).

Footnote: GPS localisation : Suzon, 47°24'14.45"N, 4°53'1.46"E ; Romaine, 47°31'53.88"N, 5°53'4.15"E ; Vèze, 47°14'1.42"N, 5°34'37.69"E ; Ouche, 47°17'54.56"N, 5°2'21.97"E ; Serein, 47°27'58.15"N, 4°7'42.20"E ; Seine, 47°31'53.72"N, 4°41'42.12"E ; Source, 47°20'57.4"N, 4°47'56.70"E ; Morte, 47°26'48.30"N, 5°41'56.52"E ; Résie, 47°19'28.54"N, 5°32'23.20"E ; Brizotte, 47°12'17.30"N, 5°26'32.19"E.
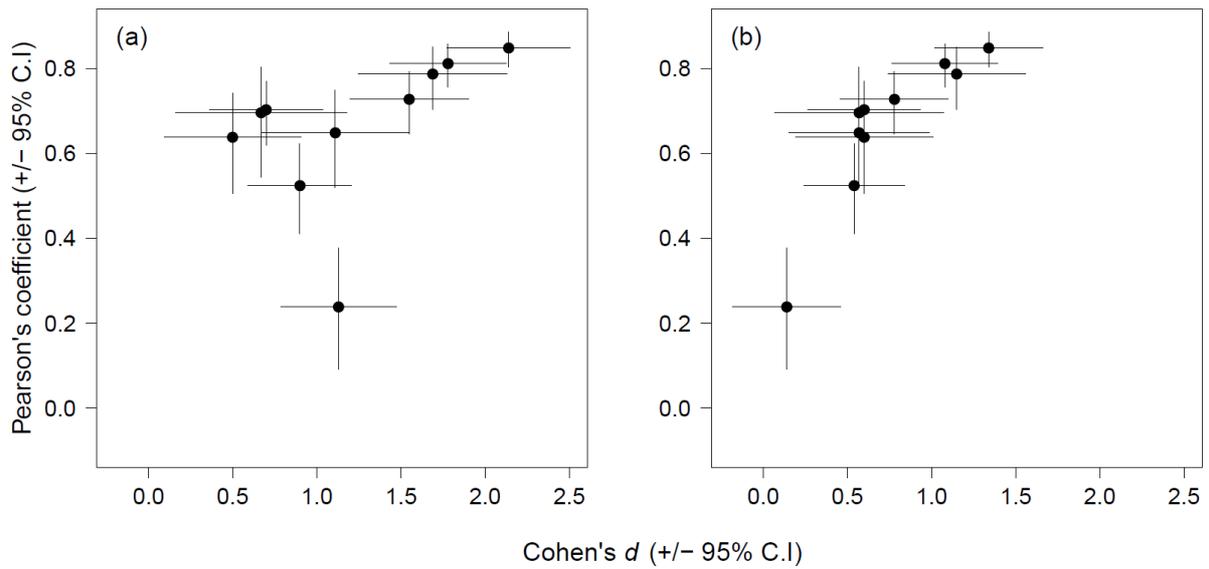
Figure 3. Observed pattern of overall size-assortative pairing within rivers (Pearson's coefficient) as a function of the difference in individual body size between sympatric MOTUs. Cohen's *d* values account for the difference in (a) male and (b) female body size between MOTUs.

Chapter 4

Sexual conflict over guarding duration: when pairing decision is not solely made by the male

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1. Gender biases and sexual conflict research

In their recent paper, Karlsson-Green and Madjidian (2011) argued that sexual conflict research is subject to biases regarding the relative role of sexes in the conflict outcome. More precisely, they surveyed terms used to refer to males or females in 30 well-cited articles from the literature on sexual conflict. They concluded that male traits are almost always referred to in "active" terms whereas females are often considered "reactive" to male traits. This emphasizes the gender bias occurring in sexual conflict research leading researchers to consider that females generally suffer from males' adaptation to mating.

Karlsson-Green and Madjidian (2011) also pointed out that researchers often omit potential costs for males which are expected under antagonistic co-evolution resulting from sexual conflict. Because male traits are thought to be harmful to females, the resulting sexual conflict should favour the evolution of female traits to avoid these costs (Arnqvist & Rowe 2005). Karlsson-Green and Madjidian (2011) claimed that female adaptations can also potentially be harmful to males. According to the authors, these costs for males are not

sufficiently recognized in the sexual conflict literature, especially in theoretical works (but see Perry & Rowe 2012).

Biases described by Karlsson-Green and Madjidian (2011) may only apply to studies where co-evolutionary arm race is already described between sexes. For this arm race to exist, males and females must be in conflict over the outcome of the expression of a trait in one of the two sexes (Chapman et al. 2003). In addition, demonstrating the very existence of a sexual conflict may also be subject to gender biases.

Sexual conflicts usually result from adaptations that are not primarily intended to harm mating partners (Parker 1979). In most studied cases, sexual conflict comes from individual adaptive responses to competition over mating or fertilisation (Chapman et al. 2003). Males sometimes evolve traits in response to sperm competition that indirectly harm females (reviewed in Stockley 1997). For instance, mating plugs that males insert after mating in certain species have evolved to secure paternity when several males competitor can mate with a given female (e.g. in *Bombus terrestris*, Sauter et al. 2000). Although this is beneficial for males in terms of male-male competition over fertilization, it also indirectly lowers female's fitness by hindering her mating rate. However, male adaptations to competition may not necessarily incur costs for females: they can have no effect on female fitness or even be beneficial for them. That is why when looking for a sexual conflict associated with the expression of a trait in one sex, it is of great importance to conduct a full economic survey of costs and benefits for males and females associated with this trait (Chapman et al. 2003, Fricke et al. 2009). Maybe because of gender biases in sexual conflict research, benefits for females associated with males adaptations have been overlooked in previous studies.

This particularly applies to mate guarding systems where male's optimal guarding duration has often been considered to be greater than female's interest. However, a few studies have shown some potential benefits for females of being guarded for a long time before or after copulation. In field crickets, males perform postcopulatory mate guarding of female, hence limiting her access to rival males. Although this behaviour has long been consider to promote sexual conflict over guarding duration, females have been shown to gain benefits in terms of reduced predation risk while being paired with a male (Rodríguez-Muñoz et al. 2011). Authors suggested that mate guarding evolved as a cooperative strategy rather than a conflict. Another example comes from the fiddler crab *Uca annulipes*, where males help their neighbouring females, with whom they mate, to defend their territory against male intruders (Milner et al. 2010). Again, this form of precopulatory guarding is thought to evolve through mutual benefits it confers to males and females.

One hallmark of sexual conflict over precopulatory mate guarding duration comes from observations of female resistance to males early attempts to initiate precopula (Ridley & Thompson 1979, Shuster 1981, Jormalainen & Merilaita 1993, 1995, Cothran 2008a, Benvenuto & Weeks 2012). In several species, this resistance has been confirmed to reduce precopula duration, so that the resulting length of mate guarding represents either a compromised strategy between males and females optimal guarding duration (modelled by Yamamura & Jormalainen 1996, e.g. Benvenuto & Weeks 2012) or is similar to the female optimal guarding duration (Jormalainen & Merilaita 1995, Jormalainen & Shuster 1999, Sparkes et al. 2000, Cothran 2008a). In some species of mate guarding crustaceans, it is still not clear whether female resistance occurs or not. In gammarids for example, it has either been considered to be important (Ward 1984a, Hunte et al. 1985, Cothran 2008c) or weak (Birkhead & Clarkson 1980, Adams & Greenwood 1983, Dick & Elwood 1989, Jormalainen & Merilaita 1995, Sutherland et al. 2007). Absence of resistance is often interpreted as evidence for important resistance-associated costs (Yamamura & Jormalainen 1996). Females resisting guarding attempts may incur energetic costs or injuries from male harassment. Because resistance usually involves violent body flexing and sudden escapes, resisting may also be costly for the current brood females carry in their ventral pouch (Jormalainen & Merilaita 1995). Alternatively, it is also possible that no costs are associated with long lasting precopulas for females or even that they acquire some benefits out of it. This last hypothesis has rarely been tested in species where sexual conflict is thought to occur. However, we believe that testing for female benefits associated with precopulatory mate guarding is a prerequisite to any attempts to describe sexual conflict and resulting antagonistic coevolution between mates (Chapman et al. 2003).

In the next section, I will review evidence for costs and benefits for males and females displaying long lasting precopulatory mate guarding.

2. Described costs and benefits of precopulatory mate guarding for males and females

Costs of precopulatory mate guarding are usually considered to be associated with energetic deprivation due to guarding and/or higher predation risk while paired. These costs are likely to be incurred by both sexes during precopula. I will first present experimental

evidence for energetically costly precopula for males and females before dealing with predation risk incurred by couples compared to unpaired individuals. In a further section, I will present more sex-specific costs and benefits that females potentially experience while pairing.

2.1 Energy expenditure in precopula

Although males may receive great competitive benefits from guarding for a long time, they have often been thought to incur also costs associated with mate guarding. Quantifications of energy compounds have revealed that males found unpaired in the field had lower energetic reserves than males found paired in the field (Sparkes et al. 1996, Plaistow et al. 2003). It has been proposed that unpaired males had just terminated an energy-demanding precopula period hence explaining their low energy reserves. However, when testing for such energetic costs in the laboratory, no costs were found associated with long lasting precopula (Jormalainen et al. 2001, Plaistow et al. 2003). In addition, no difference was found between paired and unpaired males feeding propensity in the lab, suggesting that precopula does not impede male's energy intake (Sparkes et al. 1996, Benvenuto & Weeks 2012). Similarly, females have not been shown to incur an energetic cost during the guarding phase *per se*, but rather, seemed to spend energy in antagonistic interactions with males prior to precopula (Jormalainen et al. 2001, but see Cothran 2008c). In certain species of clam shrimps, females do not exist and males guard hermaphrodites in precopula (Benvenuto et al. 2009). Hermaphrodites have been shown to experience a reduced feeding behaviour while paired which led to reduced food intake (Benvenuto & Weeks 2012). Reduced energy intake has also been put forward to explain limited growth rate of females suffering longer lasting precopula compared to females pairing for shorter times in the skeleton shrimps *Caprella penantis* (Takeshita et al. 2011).

Even though no direct energetic costs have been observed for males in precopula, it is hard to think about any energetic benefits for them associated with carrying a female for a long time. However, females may, in certain situations benefit from being paired as they do not seem to participate in couple's locomotion and may therefore spend less energy in swimming (Adams & Greenwood 1983).

2.2 Predation risk in precopula

Because they are conspicuous and less mobile, pairs are often assumed to experience a greater predation risk compared to unpaired individuals (Jormalainen 1998). This is true for a gammarid species of the genus *Hyalella* where precopula pairs are more often attacked and consumed by bluegill sunfishes predators (*Lepomis machrochirus*) compared to unpaired individuals (Cothran 2004). However, pairs are less subject to predation by dragonfly's larvae than unpaired individuals because they represent a prey too large relative to predator's size (Cothran 2004, 2008a, Cothran et al. 2012). Looking only at male susceptibility to predation, Verrell (1985) also showed that paired males were less consumed by newts than unpaired males. It is yet difficult to link these laboratory observations to actual relative predation risk of paired and unpaired individuals in the field and to its effect on optimal guarding duration for males and females. One can think of three main scenarios. First, if precopula decreases the risk of predation for both males and females (e.g. dragonfly predation, Cothran 2004) females should, like males, tend to prefer long lasting precopula, hence limiting sexual conflict over guarding duration. Second, if both males and females incur a strong predation risk while being paired, males should, like females, tend to prefer short precopula which may also limit sexual conflict over guarding duration. This situation seems to be supported as studies showed that perceived predation risk decreased male's pairing propensity (Dunn et al. 2008, Ahlgren et al. 2011). Third, it is possible that precopula actually increases predation risk for females while it decreases it for males, hence strengthening sexual conflict over guarding duration (Cothran 2004). Unpaired males are subject to strong scramble competition for access to females and should actively search for them. This may render them more subject to predation when they are unpaired compared to when they are paired. On the other hand, unpaired females should remain hidden under refuges (but see Ahlgren et al. 2011) and be less subject to predation compared to when they are paired.

2.3 Sexual cannibalism

Apart from energetic costs and predation risk, females have often been assumed to suffer sexual cannibalism while in precopula (Jormalainen 1998). Dick (1995) tested the conditions under which sexual cannibalism of females by males occurred in two species of gammarids, *Gammarus pulex* and *Gammarus duebeni*. Males almost exclusively cannibalised

newly moulted females, probably because their cuticle was soft enough to allow easy ingestion. Cannibalism of intermoult females was rare, only occurring when males were starved for several days. This therefore calls into question the importance of such cost on the occurrence of sexual conflict over guarding duration in these species.

Overall, experimental evidence argue in favour of a sexual conflict over guarding duration in mate guarding crustaceans (Jormalainen 1998). Females may suffer more costs than males associated with pairing for a long time because of possible reduced food intake or increased predation risk. However, very few studies acknowledged potential benefits for females being paired although they may be important in determining the strength of the sexual conflict over precopula.

3. Potential benefits for females

3.1 Female mate choice

Boundaries between mate choice and adaptations to sexual conflict are unclear (Chapman et al. 2003). Female resistance to mating can often be viewed as an adaptation to avoid male imposed costs or as a way for the female to discriminate between potential partners. These two processes may yet have conflicting effects on female fitness. Accepting a good quality male may involve greater cost associated with mate guarding compared to mating with a low quality male. For example, larger males have often been described to guard for longer times compared to smaller ones (Ward 1984a, Elwood & Dick 1990). If larger males are also of better quality, there may be a trade-off for females between accepting a good quality male and resisting early precopula attempts.

Until recently, no benefits for females associated with pairing with larger males had been described in mate guarding crustaceans. However, Cothran (2008c) showed that females in precopula with larger males received direct and indirect benefits. Females were less subject to dragonfly or fish predation while being paired with large males (Cothran 2008c, Cothran et al. 2012). In addition, they produced larger sons who enjoyed an advantage over smaller ones in getting access to mates. If these benefits outweigh potential costs of precopula, females

may not resist early guarding attempts when performed by large males, hence reducing the potential for sexual conflict over guarding duration.

It is worth noting that any direct benefits females receive from being paired may indirectly benefit the young they carry in their brood pouch. By protecting females against predation for instance, males might therefore also protect another male's offspring (as offspring that a female carries in her brood pouch during precopula were sired at the previous moult). Nothing is known about males' cannibalistic behaviour towards young gammarids in their partner's brood pouch. However, females may invest energy in parental care toward these young at the expense of eggs they simultaneously produce for the next reproduction with the guarding male. In that case, males should eat these young before guarding a female.

3.2 Sperm limitation avoidance

Because of their short period of sexual receptivity, females have limited opportunity to reproduce during their lifetime. Every wasted reproduction comes at a great fitness cost for them. To make sure that every egg they produce will be fertilized when they are receptive for copulation, they should avoid mating with sperm limited males. Males invest about 50% of their sperm reserve at each reproduction and have a rather long sperm replenishment time that can reach 6 days in certain species (e.g. in *G. pulex*, Lemaître et al. 2009). However, sperm depletion does not seem to affect male propensity to enter in precopula (Lemaître et al. 2009, manuscript 5). Female thus incur a severe fecundity cost when mating with a sperm depleted male (Dunn et al. 2006, personal observations). In certain species, female resistance has been suggested to play a role in discriminating between sperm depleted and non-sperm depleted males. Newly mated males are likely to be also energy depleted and therefore unable to overcome female resistance to enter into precopula (Sparkes et al. 2002). Another efficient manner for females to prevent fecundity cost due to sperm limitation could be to accept early precopula initiation. This would ensure that sperm depleted males would have the time to replenish their sperm stock before female's moult. In that case, females would not resist early precopula attempts, hence lowering sexual conflict over guarding duration.

3.3 Female reproductive rate

Males endure reduced mating opportunities by pairing up for a long time. On the other hand, females mating rate is directly linked to their moulting cycle as they can only reproduce at the time of their moult. At first glance, female mating rate seems therefore rather fixed, only decreasing with female size (the larger is a female, the longer lasting is her moulting cycle and the lower is her mating rate). However, the duration of moulting cycle is flexible as it also depends on several environmental abiotic factors such as temperature or photoperiod (Sutcliffe 1992). Individuals can also adjust their moulting depending on the biotic environment. For instance, they can delay their moult under strong pathogen prevalence because moulting makes them more susceptible to infection (Moret et al. 2010). Males can also adjust their moult depending on their female moulting date (Ward 1984b). In manuscript 4, we tested for an effect of male precopula duration on female's intermoult duration. We measured female's intermoult duration under 3 different situations: (i) when females were housed with a male in precopula in a cup, (ii) when females were housed with a male that could not perform precopula and (iii) when females were alone in the cup. Females paired with a male had significantly shorter intermoult duration compared to unpaired females. In addition, intermoult duration further decreased when females engaged in early and long lasting precopula. Intermoult duration did not affect female fecundity. Because females with shorter intermoult have greater potential reproductive rate, this provides evidence for a possible benefit for females in terms of mating rate when engaging early in precopula and argues against a strong sexual conflict over precopula duration.

4. Conclusion

When studying sexual conflict over the outcome of male adaptations to competition, there is need for a precise evaluation of costs and benefits in both males and females. Only that way could we conclude in favour of the presence of a sexual conflict or not. In mate guarding crustaceans, measurement of precopula costs and benefits for both males and females are still scarce in the literature. Potential benefits for females associated with precopula have especially been overlooked, perhaps because of gender biases in sexual conflict research. Consequently, sexual conflict may not exist in every species displaying

precopulatory mate guarding. Alternatively, long lasting precopulatory mate guarding could be viewed as resulting from cooperation between the sexes rather than conflict.

We should also be cautious when applying to our study systems concepts gathered from the study of other organisms. For instance, sexual conflict has been expensively studied in water striders (Rowe et al. 1994, Watson et al. 1998). In that system, males ride females on their back in a postcopulatory mate guarding. Water striders live on the water surface. Because of gravity, it is costly for females to carry a male for a long time (Watson et al. 1998). Such constraints may not apply to crustaceans which live underwater. In that case, it is more likely to be the male that carries the female who should not spend much energy while being held (Adams & Greenwood 1983). No studies have measured energy reserves of paired relative to unpaired females under natural conditions of current flow (but see Jormalainen et al. 2001), although it could reveal potential benefit for females associated with mate guarding.

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Does foreplay matter? *Gammarus pulex* females may benefit from long-lasting precopulatory mate guarding

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Precopulatory mate guarding (PCMG) is generally assumed to be costly for both sexes. However, males may gain by displaying long-lasting mate guarding under strong male–male competition. Surprisingly, the potential for females to benefit from being held by males has been largely overlooked in previous studies. In *Gammarus pulex*, an amphipod crustacean, PCMG lasts several weeks, yet females are described as bearing only cost from such male mating strategy. We investigated potential female benefits by assessing the effect of mate guarding on her intermoult duration. Unpaired females had longer intermoult duration than paired females. Intermoult duration clearly decreased when paired females engaged in early and long-lasting mate guarding. In addition, short intermoults and long-lasting mate guarding had no effect on egg number. These results highlight a potential benefit associated with PCMG for *G. pulex* females, suggesting that the strength of an intersexual conflict over its duration may be overestimated.

Keywords: amplexus; precopula; intermoult duration; sexual conflict

1. INTRODUCTION

In many crustacean species, males are in competition for access to females which are only receptive for a short period of time following their reproductive moult. This, associated with a lack of female reproductive synchrony, is assumed to be responsible for the evolution of precopulatory mate guarding (PCMG, also called precopula or amplexus [1]) during which a male generally grabs a female. This behaviour is considered to be costly for both sexes although some costs are sex-specific. Males, for instance, endure reduced mating opportunities (reviewed in Jormalainen [2]), reduced foraging efficiency [3], higher drift in currents [4] and injuries resulting from interference with other males [5]. Females, on the other hand, suffer increased cannibalism by males [6]. Other costs are endured by both sexes and may have limited effects on fitness asymmetry between sexes. Most notably, higher predation risk [7] and energy deprivation [5,8] are expected when paired. More obvious fitness benefits related to

long-lasting PCMG exist, but, to our knowledge, are solely described for males. In populations with a male-biased operational sex-ratio [9] and, thus, a strong male–male competition, early guarders gain mating advantages [1,10]. Surprisingly, no study has thus far investigated potential fitness benefits for females engaged in long-lasting amplexus. As a consequence, males are assumed to display long amplexus to ensure copulation, while females are presumed to prefer short precopula to avoid associated costs [2]. Consequently, PCMG is typically assumed to lead to intersexual conflict over its duration [11].

In *Gammarus pulex*, a freshwater amphipod, a male guards a female during her intermoult (time between two moults) before she becomes receptive for copulation. Species exhibiting strong intersexual conflict over PCMG duration are usually characterized by short PCMG periods and female resistance to males' early guarding attempts [2]. On the contrary, *G. pulex* amplexus durations are surprisingly long-lasting (up to 20 days), while females do not seem to exhibit any adaptations to shorten it [12]. Most studies have typically focused on costs incurred by females (reviewed in [2]), thereby ignoring the possibility that long-lasting PCMG may also be beneficial for them. This study explores the potential benefits of PCMG for *G. pulex* females. We tested for PCMG effects on female intermoult duration (i.e. delay between two reproductions) and discussed our results in the context of intersexual conflict over precopula duration.

2. MATERIAL AND METHODS

Using kick sampling and a hand net [13], individuals were collected once a week between 18 March and 22 April 2009 in the Suzon river in Burgundy (N: $47°24,215'$; E: $4°52,974'$) and immediately taken to the laboratory. Couples were isolated and maintained in 100 ml cups under a constant photoperiod (12 : 12 h) in ultraviolet-treated water at 15°C. Five days after moult, each female was assigned to one of the three treatment groups: (i) 121 females were individually put with a male previously in PCMG with a different female; (ii) 22 females were housed with a male (previously in PCMG) that had had its gnathopods removed, thereby preventing amplexus [14]; and (iii) 42 were placed alone in cups. These three treatment groups were maintained simultaneously in the controlled conditions described above. Each cup was checked daily for female moult by the presence of an exuvium. Intermoult duration (number of days between two consecutive moults), number of days spent in PCMG and egg number were recorded. Male and female body size were estimated after female moult by measuring the fourth coxal plate [15] using a Nikon SMZ-10A stereoscopic microscope and a VTO 232 video-measure system from Linkam Scientific Instruments Ltd.

Every female moulted during the experiment. Among the 121 females of the first treatment, only 105 engaged in amplexus. For the purpose of statistical analysis, females were assigned to one of three categories: females (P) observed in precopula for at least one day ($n = 105$); females (NP) with a male never observed in precopula ($n = 38$); and single females (S) ($n = 42$). Females from the three categories did not significantly differ in size ($F_{2,182} = 2.96$, $p = 0.054$) or date of collection ($\chi^2_{1,183} = 0.41$, $p = 0.52$).

A Cox proportional hazards regression [16] was performed to assess the effect of the three categories and female size on female intermoult duration. PCMG was considered to be discontinuous when the female was observed at least one time alone since the beginning of PCMG. Using P females solely, a second Cox regression assessed the effect of female size, continuous nature of PCMG, with either time to first PCMG or PCMG duration fitted as covariates, on female intermoult duration. Schoenfeld residuals were examined to assess proportional hazards assumption of the Cox regressions [17]. We used a multiple linear model to test for the effect of male and female size, with either intermoult duration, time to first PCMG, PCMG duration or continuous nature of PCMG fitted as covariates, on female egg number. Homogeneity of variance was verified using a Bartlett test. A Shapiro–Wilks' test was performed to assess the normality of the residuals.
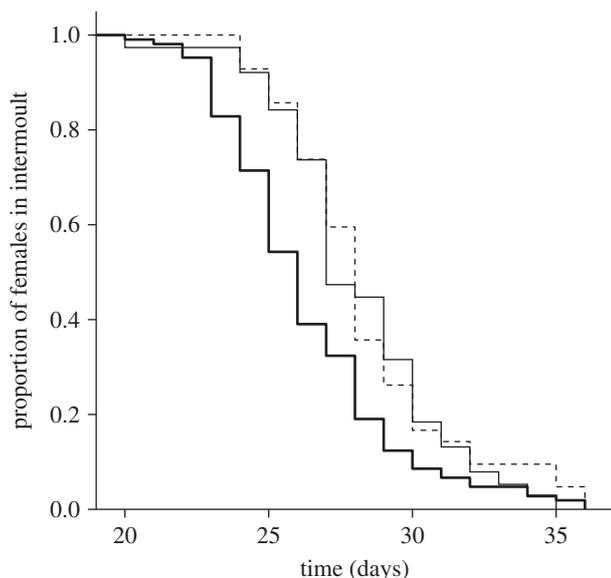
Figure 1. Proportion of females in intermoult as a function of time for the three categories of females. Bold line: P, females in precopula ($n = 105$); thin line: NP, females with a male without precopula ($n = 38$); dashed line: S, single females ($n = 42$).

3. RESULTS

Larger females showed longer intermoults ($\chi^2_{1,183} = 13.12$, $p < 0.001$). There was also a strong effect of female categories (P, NP or S) on their intermoult duration (Cox regression, $\chi^2_{2,182} = 14.86$, $p < 0.001$; figure 1). Intermoult duration of females in PCMG (26.3 ± 3.1 days) was on average 2 days shorter than those of S females (28.4 ± 3 days, contrast post hoc test, $z = 2.63$, $p < 0.01$) and NP females (28.1 ± 3 days, $z = 3.32$, $p < 0.001$) which showed similar intermoult duration ($z = 0.77$, $p = 0.44$). Everything else being equal, females displaying PCMG increased their probability of moulting by 62 per cent (exponentiated regression coefficient 1.62) when compared with single females.

P females spent on average 7.1 ± 3.4 days in PCMG (range: 1–18). Sixty-one per cent of females were guarded without interruption, but there was no difference in total time spent in PCMG between females engaged in continuous or discontinuous PCMG ($F_{1,103} = 0.98$, $p = 0.32$). The continuous or discontinuous nature of PCMG did not have an effect on female intermoult duration ($\chi^2_{1,103} = 0.56$, $p = 0.45$). Female intermoult duration was shortened by both early PCMG initiation ($\chi^2_{1,103} = 28.75$, $p < 0.001$) and long-lasting PCMG ($\chi^2_{1,103} = 5.79$, $p < 0.05$) but there was no effect of male size ($\chi^2_{1,103} = 1.16$, $p = 0.28$). On the other hand, the longer the time before first amplexus, the shorter the PCMG ($F_{1,103} = 87.6$, $p < 0.001$). Larger females carried more eggs in their brood pouch after fertilization than smaller ones ($F_{1,103} = 19$, $p < 0.001$) and none of the other variables tested had an effect on egg number (intermoult duration: $F_{1,103} = 0.59$, $p = 0.441$; PCMG duration: $F_{1,103} = 1.3 \times 10^{-5}$, $p = 0.99$; PCMG continuous nature: $F_{1,103} = 0.41$, $p = 0.52$; time to first PCMG: $F_{1,103} = 0.14$, $p = 0.70$; male size: $F_{1,103} = 0.90$, $p = 0.34$).

4. DISCUSSION

Paired females showed shorter intermoults when compared to unpaired ones. In addition, their intermoults were shortened by both early and long-lasting PCMG, without decreasing the number of eggs laid. Intermoult duration directly reflects female reproductive time-out in *G. pulex*. Females with short intermoults have higher mating rates. Early and long-lasting PCMG may then confer potential benefits on females. Further investigations are still required to clarify to what extent it is related to higher lifetime reproductive success. More generally, it is still not clear whether high mating rate contributes positively to overall female fitness [18]. We do not know if PCMG has an effect on other female components of fitness such as survival or fecundity. It could have a deleterious effect on vitellogenesis and thus on offspring number and survival [2]. However, in our experiment, PCMG had no effect on egg number, which is the first assessment of *G. pulex* female fecundity. A recent study on another amphipod species even suggests that pairing behaviour could reduce predation risk [7]. More generally, these results are consistent with previous findings of Ward [19]. Early PCMG has also been shown to shorten female intermoult duration in other crustacean species (*Armadillidium vulgarae* [20], *Paracerceis sculpa* [21] and *Eogammarus oclairi* [22]), although there is no evidence whether it has an effect on female reproductive success.

Potential benefits gained by female *G. pulex* during PCMG argue against a strong intersexual conflict over precopula duration, as both males and females should favour early and long-lasting PCMG. Besides, in species where a clear conflict exists, females generally resist early guarding attempts [23]. In *G. pulex*, resistance displayed by females has been interpreted as a form of mate choice [24,25] rather than a way to shorten PCMG duration [12,26]. We suggest that future studies should focus on females' potential benefits from long-lasting PCMG. Precise evaluation of fitness costs and benefits for both sexes, especially regarding energy intake of paired and single individuals, should clarify the potential for intersexual conflict over PCMG duration.

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General conclusion

1. Main findings

1.1 Male mate choice and size-assortative pairing

Precopulatory mate guarding in crustacean has exclusively been studied by considering males adaptations under strong sexual selection imposed by females' short period receptivity to copulation. Consequently, temporary mate guarding have only been thought to either result from male differences in competitivity within populations (Härdling et al. 2004) or from females resistance to early precopula attempts in response to the sexual conflict over guarding duration (Yamamura & Jormalainen 1996). Considering precopulatory mate guarding as a male mate choice strategy, we showed that temporary mate guarding observed in natural population can result from male mate choice based on multiple criteria of female's quality. Contrary to previous studies, we predicted that if females vary strongly in fecundity within a population, males should discriminate between potential females before entering in precopula even under balanced or male-biased sex ratio.

However, male choosiness was never very strong when males encountered females sequentially. Under the strong male-male competition imposed by female's short period of sexual receptivity, males only rarely forego a mating opportunity to continue searching for a better partner. We therefore suggested that in species presenting long lasting association of partners before mating, mate choice could happen after initial pair formation. Male may first pair with the first encountered female before potentially switching partners when an unpaired female is at proximity to the couple. This situation also presumably facilitates comparison of females' quality because it allows simultaneous female encounter (Bateson & Healy 2005). However, our results showed that in an amphipod crustacean, males did not make switching decision after comparing the quality of the two females. Instead, they tended to switch females when their own partner was of low quality regarding her size and her time left to

moult. We suggested that mate guarding can function as a sampling strategy thanks to which males assess female's quality before deciding to reject or accept her. With poor information about unpaired available females' quality, males could base their decision rule on previous mating experiences as it has been reported in another amphipod species (Hunte et al. 1985). This result highlights the possibility that individuals employ rules of thumb in mate choice decisions. They may use only a fraction of the information available, especially when more precise assessment of mate's quality is difficult and/or subject to errors (Hutchinson & Gigerenzer 2005). Theoretical investigations are needed to understand the adaptive significance of such rules of thumb under constraints on assessment.

These two results emphasize the difficulty of inferring pairing patterns from mating preferences only, without considering the underlying pairing process. It is still not clear whether, in mate guarding crustaceans, male mate choice based on body size can lead to a pattern of size-assortative pairing. The lack of support for the capacity of males to discriminate females on size under strong competition for pairing argues against this hypothesis. However, we did not explicitly model the influence of male mate choice on female's size on size-assortative pairing. In addition, it is possible that a difference in competitive ability between males could have an effect on their capacity to exert a mate choice under strong competition. Future investigations should test this possibility and its effect on the occurrence of size-assortative pairing in natural populations.

We suggest that future studies on size-assortative pairing should also focus on the role of other type of mating preferences to explain it. State-dependent valuation of partners, for instance, considerably lowers competition for preferred females as preferences depend on male's own quality and is thus not shared by every male in the population. The female-sooner norm as a male guarding criterion for mate choice represents a novel, but not exclusive cause for size-assortative mating in mate guarding crustaceans. It shows for the first time that a decision rule based on time left to moult can translate into a pattern on size. This illustrates the concept of equifinality which states that several preferences can lead to the same pattern and emphasizes the importance of avoiding inferential fallacies in studies of assortative mating (Burley 1983).

1.2 Sexual conflict over precopulatory mate guarding duration

It is difficult to determine whether conflict occurs in every species presenting long lasting precopulatory mate guarding. Costs for females associated with precopulatory mate guarding have been observed (e.g. Cothran 2004, Takeshita et al. 2011). However, such examinations may be incomplete. Research on sexual conflict may be subject to gender biases which hamper investigations of costs and benefits associated with precopula for males and females. Perhaps because precopulatory mate guarding is supposed to be a coercive male behaviour, females are usually assumed to suffer only costs in precopula. However, precopulatory mate guarding may also benefit females in certain cases. We showed that females who spent longer times in precopula had shorter intermoult duration compared to females alone during their moulting cycle. Shorter intermoult may be beneficial for females as it may provide them with more reproductive events over their life time. Further studies are yet needed to understand the life time fitness consequences of long lasting precopula for females.

Although precise economical investigations are necessary to understand the outcome of male traits on female fitness, it may be difficult to spot individual's characteristic affected by males' phenotype. Because we partially base our investigations on previous work done in different species, under different ecological conditions, we may have preconceived ideas about which potential costs or benefits should be tested to characterize a sexual conflict. Besides, measures of costs and benefits made in the lab may not always reflect the real effect on fitness of a given male's behaviour in the field. For example, predation risk measured in the lab of paired and unpaired females gammarids are often made without considering the distribution of paired and unpaired individuals in the river (e.g. Dunn et al. 2008). Several studies have reported that pairs are more predated than unpaired individuals in the lab (e.g. Cothran 2004, Cothran et al. 2012). However, other studies suggested that couples are less mobile and tend to stay hidden under rocks, hence being presumably less subject to predation (e.g. Sparkes et al. 1996). When it could appear to be costly in the lab to be paired, it is actually beneficial in the field.

Even when precisely measuring costs and benefits for each sex associated with males' behaviour, it is difficult to interpret them over a few reproductions only. Evidence of potential female benefits associated with male traits should be interpreted with caution. If females gain benefits directly from the initial male adaptation to competition, this could argue against the

occurrence of sexual conflict. However, benefits can also indicate a female adaptation in response to sexual conflict. In that case, females might make "the best of a bad situation". For instance, in the damselfly *Hetaerina americana*, males harass females for mating which has been showed to reduce their survival. Females are also more fecund when harassed. This has been interpreted as a female compensatory response to reduced future expectations of reproduction (Còrdoba-Aguilar 2009).

Because of these possible biases in interpretation, it is difficult to interpret the function of female traits. Darwin (1871) interpreted males' clasping organs as adaptations to facilitate reproduction for the mutual benefit of both males and females. However, these organs can also be seen as adaptations to sexual conflict over female monopolization (Arnqvist & Rowe 2005). Similarly, it is difficult to interpret specialized sites for male grasping on female's cuticle in mate guarding crustaceans (Platvoet et al. 2006). These could be viewed as adaptations to facilitate copulation during the short window of female receptivity. Alternatively, it can be interpreted as a female adaptation to conflict to avoid injuries due to males grasping behaviour.

2. Prospects

2.1 Male mate choice and sexual conflict

One possible future direction for research in sexual conflict could be to investigate the effect of male mate choice on the intensity of sexual conflict over different female phenotypes (Bonduriansky 2009). In species with sexual conflict over precopulatory mate guarding duration for instance, preferred females may suffer long lasting precopula or frequent attempts to form precopula. If females vary substantially in quality, males are predicted to prefer to consort with larger females, even when they are far from reproduction. On the other hand, they would reject smaller females unless they are really close to reproduction. In that scenario, smaller females suffer minor costs associated with precopulatory mate guarding, hence lowering the potential for sexual conflict between males and smaller females. However, larger females might suffer high costs associated with long lasting mate guarding which may strengthen the sexual conflict over guarding duration between males and large females. In

gammarids, larger females have often been described as being more prone to display resistance behaviour (Ward 1984a, Hunte et al. 1985).

It is worth noting that in that scenario, male mate choice would probably not lead to variation in female mating success. Small females do not mate less than larger females as they are eventually chosen when they are close to reproduction. Male mate choice should therefore impose a selective pressure on females to avoid long lasting mating, instead of enhancing sexual selection on females. Contrary to female mate choice, male mate choice should, in that case, lead to the evolution of female resistance instead of leading to the evolution of females' ornaments and displays to be chosen as a mate.

2.2 Male selfish behaviours and sexual conflict

There is a possible conceptual analogy between researches carried out on sexual conflict and on other well-known social interactions leading to antagonistic co-evolution such as host parasite interactions. In both, the fitness of at least one of the actor depends on the interaction. Parasites need their host to survive or mate; mating partners need one another to reproduce. In such situations, selection would probably favour adaptations that do not harm too much the other actor of the interaction. Too virulent parasites may kill their host, hence dying too. Male adaptations harmful to females may kill them or decrease their fecundity, hence indirectly decreasing male reproductive success. However, when reproduction is not at stake, males and female conflict should be much harsher. Males sometimes present adaptations that function to reduce female fecundity at the expense of competitor males. For example, in the parasitoid wasps *Trichogramma evanescens*, sperm depleted males continue to mate with receptive females even though they do not sire offspring (Damiens & Boivin 2006). This behaviour reduces female ability to store other males' sperm and comes at a fecundity cost to her. Authors suggested that males may thus increase their relative fitness by decreasing mating success of competitor males (Damiens & Boivin 2006). In mate guarding crustaceans, such selfish behaviours (West & Gardner 2010) may exist if sperm depleted males mate with receptive females, hence wasting their clutch at the expense of other males. Future investigations should study selfish behaviours in species where reproduction is restricted to a short period of time, because each wasted brood comes at a strong mating opportunity cost for individuals in the population. In addition to the strong sexual conflict it creates, such selfish behaviour may thus lead to a tragedy of the common (Rankin et al. 2011).

2.3 Competition and the direction of sexual selection

Competition is the primary determinant of sexual selection and as such should be under particular scrutiny when studying mating strategies in each sex. The sex suffering the most competition for mates (usually males) evolves traits to cope with it. These traits may sometimes be costly and feedback into making individuals that express them potentially choosy. On the other hand, the sex that suffers the least competition (usually females) may evolve strong choosiness towards high quality partners.

However, let us take the case where only a small proportion of males are preferred by females. Before directly concluding that this leads to strong disparity in males mating success and strong sexual selection on males, one might have to observe how pairs form in nature. If preferred males are limited in resources needed for reproduction such as sperm or if they are preferred because they provide parental care, each time a female has access to a male, this preferred male will not be available for other females for a given duration. In that scenario, some females might have to mate with non-preferred males. As a consequence, competition in males due to female mate choice is relaxed as non-preferred males also access to reproduction. This presumably decreases difference in mating success among males in a population, hence lowering sexual selection on males and its potential to lead to the evolution of extravagant male competitive traits.

2.4 Competition and the link between preferences and mating patterns

Acknowledging the role of competition in decision making also leads to important considerations regarding the link between mating preferences and mating patterns. If mating outcomes are subject to constraints due to competition for preferred mates, individuals should for instance show mate choices which depend on the perceived level of competition. This has been suggested to happen in males displaying prudent choices (Fawcett & Johnstone 2003, Härdling & Kokko 2005, Venner et al. 2010). But this may be also true for females (Cotton et al. 2006). Female preference is only one component of female mate choice. Mate choice can also be influenced by the density, the availability or the distribution of preferred males (Widemo & Sæther 1999, Cotton et al. 2006). Competition for access to preferred males may lead females to adjust their preference towards lower quality individuals. This highlights the importance of taking into account the whole pairing process under natural conditions when

studying the effect of preferences on mating patterns (Wagner 1998, Cotton et al. 2006). The observation of mate choice in controlled environments where competition is inexistent, may not account for the way mating preferences are expressed in more natural settings (Wagner 1998).

2.5 Constraints on mating preferences

As presented above, competition may hamper the translation of mating preferences into mating patterns by constraining individual access to preferred mates. However, there can be also constraints on assessment of potential mates. Assessment and valuation of potential mates are likely to be comparative in nature (Bateson & Healy 2005). Individuals may compare several available alternative encountered simultaneously (Janetos 1980) or they may compare potential mate's quality to the quality of previous reproductive partners (i.e. Bayesian decision making, McNamara et al. 2006). However, the comparison between mates may be subject to biases. The evaluation of potential mate's quality is sometimes based on several criteria which may render their precise assessment subject to errors (Candolin 2003). In addition, mating decision may vary under different contexts, because the perception of mate's quality may depend on the other potential mates surrounding it (Bateson & Healy 2005). For example, if a female encounters a male of a given size surrounded by smaller males, she may be more willing to accept him as mate than if he was surrounded by larger males (Bateson & Healy 2005). Environmental variation in male spatial distribution in mating systems such as leks may influence female decision making and therefore influence the resulting pattern of mate choice.

On the other hand, researches in mate choice often make the strong assumption that individuals are capable of perfect assessment of the quality of potential mates before making a choice (Bateson & Healy 2005). It is supposed that the internal machinery underlying decision making allows individuals to behave optimally in every encountered situations, no matter how complex the environment (Fawcett et al. in press). However, the range of potential situation is likely to be too important for evolution to have led to optimal behaviour in all of them (McNamara & Houston 2009). Instead, under complex and uncertain environments where it is difficult to acquire perfect information about different options, individuals should possess adaptations which allow them to perform well in general. In mate choice situations, potential mates vary according to different modalities and individuals facing competition

should be able to make rapid and efficient choices. In such situations, evolution may have favoured individuals using rules of thumb for mate choice. For example, individuals may use only partial information about potential mates to make their decision, valuating partners on the basis of one criterion only when their quality actually depends on several traits, difficult to assess (Hutchinson & Gigenrenzer 2005). Individuals using rules of thumbs may not choose the best available partner every time but may achieve good mating success on average when mate's assessment is costly and subject to errors.

Similar to biases in decision making presented above, rules of thumbs may not lead to consistent patterns of mate choice across situations, rendering difficult inferences from mating preferences about mating patterns at the level of the population.

2.6 The role of competition and constraints on mating preferences in models of sexual selection

Considering the effect of competition on individual's decision rules and biases on the expression of mating preferences is of major importance in sexual selection research. In order to understand the effect of female preferences on the evolution of male traits, we have to first understand how female preferences translate into mating. In species were females compete for the access to preferred males and/or males' quality depends on multiple modalities difficult to assess, pairing process may not lead to similar patterns of mate choice in different situations. This may limit the variation in male mating success because preferred as well as non-preferred males are likely to have access to reproduction. Consequently, sexual selection on males may not be as strong as previously thought, hence limiting the evolution of costly extravagant ornaments and displays (Widemo & Sæther 1999, Cotton et al. 2006).

We believe that the potential for inferential fallacies about the effect of female preferences on mating patterns should be acknowledge in studies of sexual selection. Future empirical or theoretical investigations should explicitly take competition into account in order to predict patterns of coevolution between preferences and traits.

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Appendix 1:

Manuscript 5

Galipaud, M., Gauthey, Z. and Bollache, L. 2011. Pairing success and sperm reserve of male *Gammarus pulex* infected by *Cyathocephalus truncatus* (Cestoda: Spathebothriidea). *Parasitology*. 138:1429-1435

During these three years, I had the possibility to write an article about an experiment that had been done before I started my PhD. As this article was not directly relevant to topics tackled in the thesis, I decided not to include it in the main text. This experiment has been conducted by Zoé Gauthey under the supervision of Loïc Bollache and me. Gammarids represent intermediate hosts for manipulative parasites with complex life cycles. These parasites sometimes affect their intermediate host's behaviour in an attempt to reach a definitive host which is usually a fish or a bird that feeds on gammarids. It has been suggested that parasite infection may affect many aspects of gammarids behaviour including their pairing strategies (see references in the manuscript). Here we measured the effect of a cestode parasite infection (*Cyathocephalus truncatus*) on pairing propensity and sperm reserves of males of the species *Gammarus pulex*. We sampled males from the field in three different states: (i) males found paired, (ii) males found unpaired and (iii) males found infected. Infected males had lower sperm reserves than other males and they had a lower propensity to pair with a female compared to males found previously paired. However, males found previously unpaired in the field had also a low propensity to pair, similar to parasitized males. We discuss these results in light of parasitic manipulation and emphasise the importance of considering uninfected unpaired individuals when studying the effect of manipulative parasites on male sexual behaviour. You can find the whole detailed study below.

Pairing success and sperm reserve of male *Gammarus pulex* infected by *Cyathocephalus truncatus* (Cestoda: Spathebothriidea)

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SUMMARY

Manipulative parasites with complex life cycles are known to induce behavioural and physiological changes in their intermediate hosts. *Cyathocephalus truncatus* is a manipulative parasite which infects *Gammarus pulex* as intermediate host. *G. pulex* males display pre-copulatory mate guarding as a response to male-male competition for access to receptive females. In this paper, we tested the influence that *C. truncatus*-infection might have on male *G. pulex* sperm number and pairing success. We considered 3 classes of *G. pulex* males in our experiments: (i) uninfected males found paired in the field, (ii) uninfected males found unpaired in the field, or (iii) infected males found unpaired in the field. Both infected males and uninfected unpaired males paired less with a new female than uninfected paired males did. Furthermore, infected males appear to be at a strong disadvantage when directly competing for females with a healthy rival male, and had fewer sperm in their testes. We discuss the potential effect of male and female mating strategies on such male host mating alteration.

Key words: manipulative parasite, male-male competition, pathogenic effects, gammarids, tapeworm.

INTRODUCTION

Relationships between levels of parasitism and male mating success have received much attention over the past few decades (e.g. Hamilton and Zuk, 1982; Read, 1990; Clayton, 1991; Dunn, 2005). Three main hypotheses are usually put forward to explain the observed decrease in infected male mating success. First, parasite infection may affect male ability to compete with other males for access to reproduction and fertilization (Howard and Minchella, 1990; Forbes, 1991). Infection can alter male potential to find and secure a territory (Borgia, 1986) or a mate (e.g. in arthropods, Carmichael *et al.* 1993; Zohar and Holmes, 1998; Bollache *et al.* 2001). Infected males may also be less able to directly interfere with competitors to gain access to females (Zohar and Holmes, 1998; Bollache *et al.* 2001). Second, females may refuse to mate with infected males (Milinski and Bakker, 1990). They should prefer to consort with uninfected males to avoid contamination by parasites (Able, 1996). They should also favour males that resist parasite infection as this could provide them with pathogen-resistant offspring (Hamilton and Zuk, 1982). Third, the mating success reduction of infected male hosts may result from parasite adaptations (Hurd, 2001; Moore, 2002; Lefèvre *et al.* 2008). Parasites with complex life cycles sometimes present

strategies to increase their chance of transmission from an intermediate host to a definitive host (Poulin, 1994; Lafferty, 1999; Lagrue *et al.* 2007). In case of trophic transmission, parasites can manipulate host behaviour and physiology to make it more susceptible to predation by a definitive host (Lafferty, 1999; Lagrue *et al.* 2007). Manipulation can hence induce modifications in some aspects of host behaviour, such as general activity or spatial and temporal distribution, reducing their probability of encountering mates (Rasmussen, 1959; Thomas *et al.* 1995; Zohar and Holmes, 1998; Tain *et al.* 2006). Manipulative parasites can also modify host physiology, leading to fecundity alteration, suspension or even castration with significant effects on mating behaviour (Baudoin, 1975; Thompson and Kavaliers, 1994; Bollache *et al.* 2002; Ferreira *et al.* 2005). Most studies have focused on the influence of infected female fecundity reduction on male mating preferences (Poulton and Thompson, 1987; Bollache *et al.* 2002; Dunn *et al.* 2006). On the other hand, the effects of manipulative parasite on spermatogenesis and male mating success have been poorly documented (Bierbower and Sparkes, 2007).

Cyathocephalus truncatus (Cestoda: Spathebothriidea) is a tapeworm widespread in Europe. It almost exclusively infects amphipod crustaceans, such as *Gammarus pulex*, as intermediate hosts, and fishes as definitive hosts (Okaka, 1984). Franceschi *et al.* (2007) showed that *C. truncatus* was able to manipulate the behaviour of its *G. pulex* intermediate host. Infected individuals have been described to be

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significantly less photophobic than uninfected ones. This alteration in infected *G. pulex* behaviour makes them more conspicuous to visual predators, and explains the previously observed increase of *C. truncatus*-infected gammarid predation rate (Knudsen *et al*. 2001). In addition, Franceschi *et al*. (2007) observed various *C. truncatus* pathogenic effects, especially on intermediate host survival, swimming activity and oxygen consumption.

Mating behaviour in *G. pulex* is characterized by a pre-copulatory mate-guarding phase (also called amplexus or pre-copula) during which a male carries a female beneath his ventral surface for several days (up to 20 days, e.g. Galipaud *et al*. 2011). This mate-guarding period usually begins when the female initiates vitellogenesis and thus becomes receptive to pairing. The pre-copula ends with female moulting. The female then becomes receptive for reproduction with the guarding male for about a day (Sutcliffe, 1992; Bollache *et al*. 2000). Pre-copulatory mate-guarding behaviour is thought to have evolved as a male competitive strategy in response to this brief period of female sexual receptivity (Parker, 1974; Grafen and Ridley, 1983). In amphipods, parasite infection often correlates with a decrease in male ability to successfully pair with a female in nature (Ward, 1986; Thomas *et al*. 1995; Zohar and Holmes, 1998; Bollache *et al*. 2001). According to previous studies, several processes related to sexual selection may explain this pattern. Both female mate choice and male-male competition for females have been suggested as important components of infected males, lower pairing success (Zohar and Holmes, 1998; Bollache *et al*. 2001). The aim of this study was to combine field observations and laboratory experiments to assess the influence of *C. truncatus* on male *G. pulex* (i) sperm reserves and (ii) pairing success and competitive ability.

MATERIALS AND METHODS

Field collection

All gammarids were collected from March to May 2009 in a small tributary of River Suzon, Burgundy, eastern France (N: 47°24,215′; E: 4°52,974′) using a hand net and the kick sampling method described by Hynes (1954). The relative large worm size and its white colour, visible through the gammarid cuticle, make infected hosts easy to recognize. All infected individuals sampled in the field were only infected by one larva.

Following Bush *et al*. (1997), we estimated the prevalence of *C. truncatus* in the field by measuring the proportion of infected individuals in a first sample. For laboratory experiments, we sampled gammarids for a second time in the field (hereafter referred to as 'the second field sample'), looking specifically for infected males and uninfected individuals.

Uninfected males were either found unpaired or paired with an uninfected female. Infected males, however, were only found unpaired in the field. In this second field sample, *G. pulex* males were thus found in the following 3 different field states: (i) uninfected paired, (ii) uninfected unpaired or (iii) infected. We used males from this second field sample (paired males where separated from their previous female) either for the inclination experiment and sperm measurement ($n = 105$) or for the competition experiment ($n = 66$), as described below.

Laboratory studies

In the laboratory, gammarids were maintained under a constant photo-period (12:12 h) in well-aerated tanks containing UV-treated water at 15 °C and leaf litter. For experiments, gammarids were individually housed in small plastic cups (h = 7 cm; Ø = 9 cm). At the end of each experiment, all individuals were killed, using 70% alcohol, and measured (size of the fourth coxal plate, e.g. Bollache *et al*. 2002) using a stereoscopic microscope (Nikon SMZ 1500) and the Lucia G 4.81 software. With the same apparatus, we also measured the total body length of cestodes. No gammarids were used more than once for experiments. Individuals that moulted or died during experiments were excluded from the dataset.

Male inclination to pair. We first investigated the effect of male field states (infected unpaired, uninfected unpaired and uninfected paired) on male inclination to pair with a new uninfected female. Males were first individually allowed to acclimatize for 1 h in a plastic cup. A female was then added to each cup. All females used in this experiment had already been caught in pre-copula to control for their receptivity to pairing. Their position in their inter-moult (i.e. the time between 2 successive moults) was approximately assessed (either close to moult, in the middle of intermoult or at the beginning of intermoult) based on the developmental stage of embryos in their brood pouch (Geffard *et al*. 2010). Cups were first checked after 1 h and then after a period of 24 h to determine whether individuals had entered into pre-copula. All individuals were then measured. Males were also dissected for sperm number assessment as described below. The effect of males field states on their pairing success was tested using a logistic regression model with sperm number, female position in their intermoult, and male and female size as covariates.

Sperm reserve. We also assessed sperm reserve of males from the inclination experiment using the protocol described by Lemaître *et al*. (2009). Briefly, 1 testis per male was removed and isolated in a watch glass, in 1 ml of Crustacean Ringer solution. The gonad was cut into small fragments with fine forceps

under a binocular microscope. This allowed sperm to mix with the Ringer. The solution was then exposed to ultrawaves for 10 sec to separate sperm from membranes without damaging the gametes (Ultra-waves tank, Branson 2200 Branson cleaning Equipment Company, Shelton, Co, USA). The solution was homogenized with a micropipette (i.e. by pushing and pulling liquid for 30 sec) and $4 \times 10\,\mu$l drops per male were placed on a slide and dried for 10 min. Slides were then gently rinsed with demineralized water to eliminate Ringer's crystals before allowing them to dry again for 30 min. Sperms of each slide were counted under optic microscopy (Nikon Eclipse E600, magnification × 100). Total sperm reserve of each individual was therefore estimated by combining sperm number of all 4 drops ($40\,\mu$l). Using an ANCOVA, we tested for the effect of male field state on sperm reserve with male size as covariate. Sperm reserve data were Box-Cox transformed to meet normality. Homogeneity of variance was verified with a Bartlett test.

Male-male competition. We also studied the ability of infected *G. pulex* males to pair with a female in the presence of an uninfected competitor male. Two males of similar size (*t*-test; $t = 0.83$, $P = 0.406$), 1 infected and 1 uninfected (previously paired in the field), were introduced in a plastic cup and allowed to acclimatize for 1 h. A previously paired female (i.e. receptive for pairing) was then added to each cup. Females used for this experiment were always smaller than the 2 males in their cups. Trials ($n = 33$ replicates) were examined every hour during 1 day (i.e. 12 h). Once 1 of the 2 males had formed pre-copula, the 3 gammarids were removed from the cups and measured. After 24 h, every gammarid was removed from the apparatus. We used a binomial test to compare uninfected and infected male pairing success in competition. However, this did not distinguish between the two confounding effects of male-male interaction and male inclination to pair on male pairing probability. In order to disentangle these two effects, we also compared the pairing success of infected males in the inclination experiment (i.e. with no competition) to the pairing success of infected males in the competition experiment with a Fisher exact test. For more consistency, we also calculated the odds ratio as a measure of effect size of the difference and its 95% confidence interval (Nakagawa and Cuthill, 2007).

RESULTS

Field studies

Overall, 536 pre-copula pairs and 1113 unpaired gammarids (643 males and 470 females) were collected in the first sample. Parasite prevalence was extremely low in the field (0·23% of *C. truncatus*-infected individuals in the first field sample, $n = 5$).

Table 1. Logistic regression of pairing success in male *Gammarus pulex* in the laboratory as a function of male field state, male and female body size, time left to the female moult and sperm number

(Values of $P < 0.05$ are given in bold font.)

Variable	D.F.	Wald chi-square	P
Field state	2	10·03	**0·006**
Male size	1	0·25	0·62
Female size	1	1·39	0·24
Time to moult	1	0·08	0·78
Sperm number	1	0·745	0·39
Male size × Field state	2	0·844	0·65

Because of this low proportion of infected individuals found in this first field sample, we were unable to reliably test for a parasite prevalence difference in males (0·25%, $n = 3$) and females (0·20%, $n = 2$). For the same reason, we were also unable to test for a difference between infected and uninfected male pairing success in this first field sample. None of the infected males collected in the first field sample were paired. On the other hand, 45·6% of uninfected males were found in amplexus. In the second field sample (i.e. gammarids dedicated to laboratory experiments), males showed size differences according to their field states (Kruskal-Wallis, $\chi_2^2 = 9.72$, $P < 0.01$). Infected unpaired males ($n = 33$) were significantly larger than uninfected unpaired males ($n = 39$, *post-hoc* test: $P < 0.01$) but did not differ in size with uninfected paired males ($n = 33$, *post-hoc* test: $P = 0.69$). Uninfected paired and unpaired males did not differ in size either (*post-hoc* test: $P = 0.06$).

Laboratory studies

Male inclination to pair. Male inclination to pair with a female was significantly related to male field state, but not to female's time left to moult, number of sperm or male and female body size (Table 1). Males infected with *C. truncatus* were significantly less likely to enter into pre-copula than uninfected paired males (*post-hoc* test, $Z = -2.44$, $P < 0.05$, Fig. 1A). Similarly, uninfected unpaired males formed significantly fewer pre-copula than uninfected paired males (*post-hoc* test, $Z = -2.64$, $P < 0.01$, Fig. 1A). However, there was no difference in pairing probability between uninfected unpaired males and *C. truncatus*-infected males (*post-hoc* test, $Z = 0.14$, $P = 0.89$, Fig. 1A). Thus, among 105 individuals, uninfected males found paired in the field were more likely to pair again with a new female (70·59%) compared to uninfected males found unpaired in the field or infected males (41·02% and 42·42% respectively, Fig. 1A).

*Sperm reserve.* Total sperm reserve (i.e. the estimated number of sperm in 1 testis) was significantly
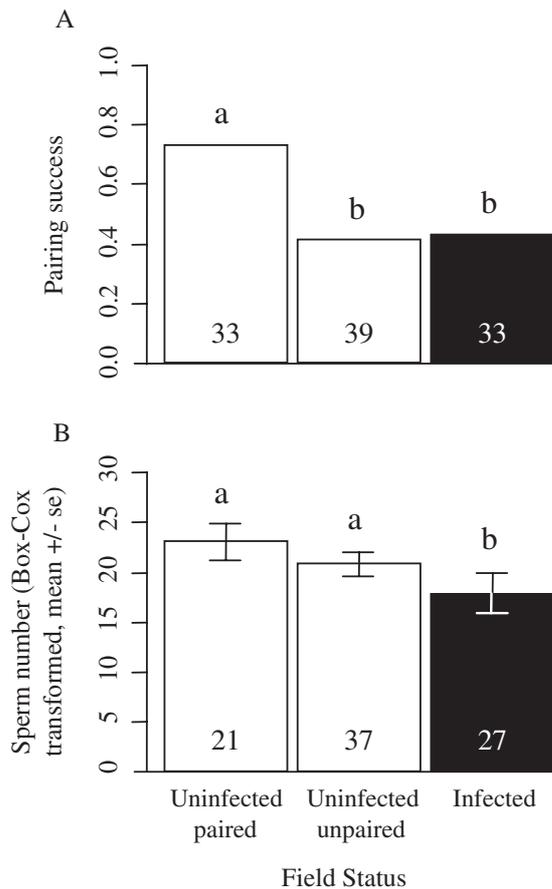
Fig.1. Pairing success (A) (proportion of males entering in pre-copula) and sperm number (B) of infected males and paired or unpaired uninfected males. Numbers inside bars represent sample sizes for each male field state. Categories sharing the same letter above their bars did not differ significantly.

Table 2. Number of parasite-infected and uninfected males that succeeded in pairing with a female in the inclination and the competition experiments

	Inclination		Competition	
Field state	Paired	Unpaired	Win	Lose
Infected	14	19	2	21
Uninfected paired	24	9	21	2
Uninfected unpaired	16	23	—	—

competitive situations, infected male pairing success was strongly decreased. Only in 2 out of 23 trials (8·7%) did *C. truncatus*-infected males succeed in entering into pre-copula when competing with an uninfected male (binomial test: $P < 0.001$). In non-competitive trials (i.e. in the inclination experiment), infected male pairing success was even significantly better than in competitive situations (odds ratio: OR = 12·78, 0·95 confidence interval ranging from 2·06 to 43·3, Fisher exact test: $P < 0.01$, see Table 2 for sample sizes).

DISCUSSION

G. pulex males exposed to *C. truncatus* infection incur a severe decline in their pairing success. Both their inclination to pair with a receptive female and their competitive ability decreased. Manipulative parasites have been reported to alter male mating success in several field-based studies (Oetinger, 1987; Zohar and Holmes, 1998; Bollache *et al.* 2001; Sparkes *et al.* 2006; Bierbower and Sparkes, 2007). In this study, no male infected by *C. truncatus* was ever found paired with a female in the field, in either of our samples. This would tend to support the pattern observed in laboratory experiments. However, the low parasitic prevalence we measured does not allow us to draw a definitive conclusion about infected male pairing success in nature. Among uninfected males, those found unpaired in the field also showed a weak tendency to pair with a new female. This is consistent with previous findings on reproductive behaviour of *G. pulex* males. They appear to show more willingness in initiating a new pre-copula after they already spent some time paired with another female (Lemaître *et al.* 2009). This suggests either that (i) a common phenomenon causes a weak paring pattern in both infected and uninfected unpaired males or, (ii) that different phenomena lead to the same difficulties in initiating pre-copula in both male states. Following the first hypothesis, it is possible that males which do not succeed in pairing with a female are also more susceptible to parasite infection. In our field samples, infected males may thus simply

affected by male field state ($F_{2,85} = 3.33$, $P = 0.04$, Fig. 1B). Infected males had lower sperm reserve than uninfected paired males (*post-hoc* test; $t = 2.296$, $P < 0.05$, Fig. 1B) or uninfected unpaired males (*post-hoc* test; $t = 2.177$, $P < 0.05$, Fig. 1B). However, uninfected paired and unpaired males did not differ regarding their sperm reserve (*post-hoc* test; $t = 0.289$, $P = 0.774$, Fig. 1B). Larger males carried more sperm in their testes than smaller males ($F_{1,85} = 6.45$, $P = 0.01$). The interaction between male size and male field state had no effect on sperm number ($F_{2,85} = 0.17$, $P = 0.84$). Among infected males, we found a positive correlation between male size and cestode size (Pearson correlation, $r = 0.63$, $n = 26$, $P < 0.001$). However, none of the following variables significantly influenced infected male sperm number: male size ($F_{1,26} = 0.79$, $P = 0.38$), cestode size ($F_{1,26} = 0.38$, $P = 0.54$), the interaction between male and cestode size ($F_{1,26} = 2.21$, $P = 0.15$).

Male-male competition. Overall, 33 assays were performed involving 2 males and a receptive female, but only 70% (23/33) resulted in a pairing. In

be weakened males, already unable to successfully pair with a female. However, the size difference we observed between uninfected unpaired males and infected males makes this hypothesis unrealistic in *G. pulex*. In the rest of the discussion, we consider the second hypothesis, acknowledging relative roles of male and female strategies and parasite manipulation to explain *G. pulex* male-mating pattern.

One hallmark of *C. truncatus* infection in males is a reduction in sperm. Such reductions have not been reported for crustaceans infected with acanthocephalan parasites: i.e. amphipod (Moore, 1984; Zohar and Holmes, 1998) or isopod (Bierbower and Sparkes, 2007). Two main phenomena could explain this effect. First, the substantial tapeworm size (up to 30% of host mass, Okaka, 1984; Franceschi *et al*. 2007) and its position in the host body cavity may induce pathogenic effects or mechanical harm on *G. pulex*, potentially resulting in reduced sperm production in infected males. This may occur either directly, by physically curtailing gametogenesis, or indirectly by acting on host nutrient availability (see Hurd, 2001 for a review). For instance, *C. truncatus*-infected gammarids have been shown to suffer a decrease in swimming activity, which may affect their foraging efficiency (Franceschi *et al*. 2007). Second, by limiting or diverting energy normally allocated to reproduction, the parasites may reduce host fecundity. Parasites often directly compete with their host for nutrients, which can reduce energy available for host gamete production. Under these conditions, a negative correlation between parasite biomass and host fecundity is expected (Hurd, 2001). In this study, no correlation was found between gammarid sperm number and tapeworm size, raising doubts about any effect of nutrient competition on host sperm reserve.

The reduced pairing of infected males could be linked to sperm reserve. But male pairing success is also expected to be affected by other parasite-induced pathogenic effects or by female mating behaviour. In this section, we consider these 3 hypotheses to explain the infected male-pairing pattern.

First, with low sperm reserves, males may change their mating behaviour, as has been suggested for other arthropod species (Kendall and Wolcott, 1999; Ortigosa and Rowe, 2003; van Son and Thiel, 2006). Uninfected unpaired males did not differ in sperm number with uninfected paired males, although they paired less often with a new female. Thus, for uninfected unpaired males, pairing propensity does not seem to be linked to sperm reserve. It would thus be surprising that the low sperm reserve in *C. truncatus*-infected males influences their inclination to pair. Lemaître *et al*. (2009) also found no effect of sperm reserve on male pairing decision in *G. pulex*.

Second, pathogenic effects induced by parasites, such as reduced swimming activity or oxygen consumption (Franceschi *et al*. 2007) may alter male-pairing success. These pathogenic effects could make struggles prior to pre-copula more difficult for infected males (Sparkes *et al*. 2006). Franceschi *et al*. (2007) also suggested that the low survival observed in *C. truncatus*-infected individuals may be due to the large amount of energy that is lost to the parasite infection. Pre-copulatory mate guarding is a long lasting and energy-expensive behaviour in *G. pulex* (Plaistow *et al*. 2003), and it is therefore possible that infected males, who may be already energy depleted, are less able to afford the energetic cost of holding a female for several days. Under these circumstances, they would not be able to pair as often as healthy males, and this could explain their low inclination to pair in our experiments. Perhaps owing to this weakened body condition, tapeworm-infected males suffered even lower pairing success when directly competing with healthy males. Our results revealed that infected males paired even less in competitive situations when compared to non-competitive situations. Evidence for such an effect of parasites on male competitive ability are scarce in the literature (Zohar and Holmes, 1998; Bollache *et al*. 2001). It is difficult to distinguish between the relative roles of interference competition *versus* scramble competition when explaining the decreased competitive ability observed in infected males. It is possible that *C. truncatus*-infected males might have had their females usurped by rival healthy males (i.e. 'take-over', Grafen and Ridley, 1983). However, take-overs are rarely, if ever, observed in *G. pulex* (Franceschi *et al*. 2010). It is thus more likely that their poorer propensity to pair resulted in a disadvantage in rapidly securing the female.

Third, female sexual behaviour would likely play a role in male pairing success. In several amphipod species displaying pre-copulatory mate guarding, females resist male guarding attempts as a form of mate choice (Elwood *et al*. 1987; Jormalainen, 1998; Cothran, 2008*a*,*b*). Male size, for instance, has been proposed to play a role in female mate choice (Wellborn and Bartholf, 2005; Cothran, 2008*a*). Our data showed that infected males were larger than uninfected unpaired males. However, they suffered an equally low mating success. If pairing is under female control, female mate choice based on male size alone does not explain the pairing pattern we observed. On the other hand, females may base their choice on other male traits such as sperm reserve. In species in which females do not store sperm, as in the case of amphipods (Hunte *et al*. 1985; Jormalainen, 1998), sperm limitation during mating can result in a fecundity cost for them (Hou and Sheng, 1999; Sadek, 2001; Sparkes *et al*. 2002, Dunn *et al*. 2006). Sparkes *et al*. (2002) demonstrated that in a stream-dwelling isopod, females avoid mating with newly mated, possibly sperm-limited males. By resisting pairing with infected males (i.e. sperm limited), *G. pulex* females may thus prevent possible fecundity costs. Female mate choice for uninfected males could

also result from other deleterious effects linked with male infection. Infected males may be of lower genetic quality (Hamilton and Zuk, 1982). Females may also risk parasite infection when mating with infected males (Keymer and Read, 1991), although *C. truncatus* horizontal transmission has never been reported between intermediate hosts. However, manipulative parasites induce behavioural and physiological changes in their intermediate host to facilitate transmission to a definitive host (Poulin, 1994). Pairing with infected individuals could thus come with a higher predation risk in intermediate host species (Sparkes *et al.* 2002). *G. pulex* have a central position in the food web as a prey of numerous fish species (MacNeil *et al.* 1999). It may then be particularly risky for females of this species to be held by a *C. truncatus*-infected male.

In conclusion, various effects related to sexual selection can explain the observed pairing success of *G. pulex* males. We observed a sperm reduction in infected males, but not in uninfected unpaired males, although they both showed a reduced pairing success. Thus, sperm number does not seem to influence male-pairing success. Rather, it seems that other infection-induced pathogenic effects related to a male's body condition may have deleterious effects on both their inclination to pair and their competitive ability. Future studies should carefully assess the influence of female mate choice, as several parasite-related deleterious effects (lower mate quality, predation risk) should alter their motivation to mate with infected males. Here, we also emphasize the importance of considering the pairing success of healthy males found unpaired in the field when studying the role of parasites on reproductive behaviour in species displaying pre-copulatory mate guarding. This provides useful cues about possible pre-existing mating bias in uninfected males, hence pondering the effect of parasite infection on host reproductive success.

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Appendix 2:

R code for the model presented in chapter 2

```r
#----------------------------------------------------------
#
# Appendix 2
# R script for the mate choice model, Chapter 2
# Basic model, finding the optimal mate choice strategy
#
#----------------------------------------------------------

rm(list = ls())

## basic parameters

lambda <- 0.1 ## encounter rate
sex_ratio <- 0.5
pop_size <- 200
time_step_to_calculate_feedback <- 0.01

nb_male <- pop_size*sex_ratio ## number of males
nb_female <- pop_size - nb_male ## number of females

tmax <- 40 ## maximum time to reproduction
m <- 40 ## maximum size
size_step <- m

beta <- 0.05

## fecundity/size distribution
## quartic function
## frequency distribution

P.s <- function(s, b = 2){
    quartic <- (s^(b)*(s-m-1)^(b))
    summation <- sum(quartic)
    result <- quartic/summation
    result
}

## initial distribution of unpaired females
t <- P.s(size)*nb_female/tmax
unpaired_female <- matrix(rep(t, tmax), nrow = size_step, ncol = tmax)


## fonction for the calculation of the beta
function_beta <- function(potential.value, l = 10){
    if(potential.value>0){
        # beta_mat <- 1-exp(-l*potential.value)
        beta_mat <- beta
    }else{
        beta_mat <- 0
    }
    beta_mat
}


## male initial mate choice
## strategy
```

```r
mutant_pref_function_matrix <- matrix(c(rep(1, size_step), rep(0, size_step*tmax-size_step))
  , nrow = size_step, ncol = tmax, byrow=T)



#############
##
## main
##
#############


##~~~~~~~~~~~~~~~~~~~~~~~~~
## the feedback functions
##~~~~~~~~~~~~~~~~~~~~~~~~~

## g is a rate at wich females change states, it is treated as a cumulative distribution
function for the exponential distribution
## 1-exp(-g)
## 40 is the number of time step we usually consider by default, with tmax=40, g corresponds
to 1 day

g <- 1

## we also consider the rates a(s, t) and b(s, t) at which males and females pair up
repectively
## they are also treated as a cumulative distribution function for the exponential
distribution
## a(s,t) = lambda*nb_unpaired_females*P(s,t)
## b(s,t) = lambda*nb_unpaired_males*P(s,t)
## it becomes 1-exp(-a(s,t)) and 1-exp(-b(s,t)) in the calculation


## calculation of the effect of male strategy on female quality distribution

previous_unpaired_female <- 100
next_unpaired_female <- 0
new_unpaired_female <- unpaired_female
former_previous_unpaired_female <- unpaired_female
check <- 1
loop_check <- 0
compteur <- 0

while(sum(abs(previous_unpaired_female - next_unpaired_female)) > 0.0001){

    time_interval <- time_step_to_calculate_feedback
    unpaired_female <- new_unpaired_female
    new_unpaired_female[,tmax]  <- (exp(-(g + (nb_male-nb_female+sum(unpaired_female))*lambda
    *mutant_pref_function_matrix[,tmax])*time_interval)) * (unpaired_female[,tmax]) + (1-exp
    (-g*time_interval))*(t)
    new_unpaired_female[,1:(tmax-1)] <- (exp(-(g + (nb_male-nb_female+sum(unpaired_female))*
    lambda*mutant_pref_function_matrix[,1:(tmax-1)])*time_interval)) * (unpaired_female[,1:(
    tmax-1)]) + (exp(-(nb_male-nb_female+sum(unpaired_female))*lambda*
    mutant_pref_function_matrix[,2:tmax] * time_interval))*(unpaired_female[,2:tmax]) - (exp
    (-(g+(nb_male-nb_female+sum(unpaired_female))*lambda*mutant_pref_function_matrix[,2:tmax
    ])*time_interval)) * unpaired_female[,2:tmax]

    compteur <- compteur+time_interval
    loop_check <- compteur
```

```r
        if(as.character(loop_check) == as.character(check)){
            ## for the loop
            previous_unpaired_female <- former_previous_unpaired_female
            next_unpaired_female <- new_unpaired_female
            ## for the next check
            former_previous_unpaired_female <- new_unpaired_female
            compteur <- 0
        }
}


##~~~~~~~~~~~~~~~~~~~~~~~~~~~
## starting value of gamma
##~~~~~~~~~~~~~~~~~~~~~~~~~~~

tlm <- 1:tmax
s <- 1:m
time_matrix <- matrix(rep(tlm, size_step), nrow = size_step, ncol = tmax, byrow = TRUE)
size_matrix <- matrix(rep(s/m, tmax), nrow = size_step, ncol = tmax)

## fecundity function
b <- 1 ## the power
a <- 1 ## the parameter to control the range
fecundity <- (size_matrix*a)^b+1

## starting value of gamma
gamma0 <- sum(unpaired_female*mutant_pref_function_matrix*fecundity)/((1/lambda)+sum(
unpaired_female*mutant_pref_function_matrix*time_matrix))

gamma_iteration <- gamma0
gamma_resident <- 100
max_diff_strat<-1
iteration <- 1
gamma_iteration_tab_calc <- NULL


## main code
while(max_diff_strat>0.000001){ ## run until gamma converges

    # the new value of gamma become resident
    gamma_resident <- gamma_iteration
    resident_pref_function_matrix <- mutant_pref_function_matrix

    ## pref_s_crit est un vecteur de valeur de s pour tte les valeur de t
    potential.value.it <- function(scrit, tcrit, resident_gamma = gamma_resident){
        pref_male <- resident_pref_function_matrix
        fecundity <- (size_matrix*a)^b+1
        pref_male[scrit, tcrit] <- 0
        best_zero <- sum((unpaired_female/sum(unpaired_female))*fecundity*pref_male) -
        resident_gamma*(1/(lambda*sum(unpaired_female))) - resident_gamma*sum((
        unpaired_female/sum(unpaired_female))*time_matrix*pref_male)
        pref_male[scrit, tcrit] <- 1
        best_one <- sum((unpaired_female/sum(unpaired_female))*fecundity*pref_male) -
        resident_gamma*(1/(lambda*sum(unpaired_female))) - resident_gamma*sum((
        unpaired_female/sum(unpaired_female))*time_matrix*pref_male)
```

```r
        sup_zero <- max(best_zero, best_one)
        if(sup_zero<0){
            best <- 0 ## this way, I can calculate the beta corresponding to 0 (which is 0)
            and it would have no consequence on the previous staretgy
            the_strategy <- resident_pref_function_matrix[scrit, tcrit]
        }else{
            if(best_zero>best_one){
                best <- best_zero
                the_strategy <- 0
            }
            if(best_zero<=best_one){
                best <- best_one
                the_strategy <- 1
            }
        }
        sortie <- c(best, the_strategy)
        sortie
}


# finding the new values of t_crit and of s_crit
female_size <- 1:m
best_response <- matrix(rep(0, size_step*tmax), nrow = size_step, ncol = tmax, byrow=T)

beta_matrix <- matrix(rep(0, size_step*tmax), nrow = size_step, ncol = tmax, byrow=T)
for(time_step in 1:tmax){
    for(s_crit in 1:m){
        best_response[s_crit, time_step] <- potential.value.it(scrit = s_crit, tcrit =
        time_step)[2]
        best_potential_value <- potential.value.it(scrit = s_crit, tcrit = time_step)[1]
        beta_matrix[s_crit, time_step] <- function_beta(best_potential_value, l = 3)
    }
}
# the mutant preference function
mutant_strategy_matrix <- best_response
beta_mutant_strategy_matrix <- (beta_matrix*mutant_strategy_matrix + (1-beta_matrix)*
resident_pref_function_matrix)
mutant_pref_function_matrix <- beta_mutant_strategy_matrix

## finding the feedback
# restart with a complete distribution of unpaireed females
size <- 1:m
t <- P.s(size)*nb_female/tmax
unpaired_female <- matrix(rep(t, tmax), nrow = size_step, ncol = tmax)

previous_unpaired_female <- 100
next_unpaired_female <- 0
new_unpaired_female <- unpaired_female
former_previous_unpaired_female <- unpaired_female
check <- 1
loop_check <- 0
compteur <- 0

while(sum(abs(previous_unpaired_female - next_unpaired_female)) > 0.0001){

    time_interval <- time_step_to_calculate_feedback
    unpaired_female <- new_unpaired_female
    new_unpaired_female[,tmax]  <- (exp(-(g + (nb_male-nb_female+sum(unpaired_female))*
```

```r
            lambda*mutant_pref_function_matrix[,tmax])*time_interval)) * (unpaired_female[,tmax])
             + (1-exp(-g*time_interval))*(t)
        new_unpaired_female[,1:(tmax-1)] <- (exp(-(g + (nb_male-nb_female+sum(unpaired_female
            ))*lambda*mutant_pref_function_matrix[,1:(tmax-1)])*time_interval)) * (
            unpaired_female[,1:(tmax-1)]) + (exp(-(nb_male-nb_female+sum(unpaired_female))*lambda
            *mutant_pref_function_matrix[,2:tmax] * time_interval))*(unpaired_female[,2:tmax]) -
            (exp(-(g+(nb_male-nb_female+sum(unpaired_female))*lambda*mutant_pref_function_matrix[
            ,2:tmax])*time_interval)) * unpaired_female[,2:tmax]


        compteur <- compteur+time_interval
        loop_check <- compteur
            if(as.character(loop_check) == as.character(check)){
                ## for the loop
                previous_unpaired_female <- former_previous_unpaired_female
                next_unpaired_female <- new_unpaired_female
                ## for the next check check
                former_previous_unpaired_female <- new_unpaired_female
                compteur <- 0
            }
    }



    # finding the new value of gamma
    fecundity <- (size_matrix*a)^b+1
    gamma_iteration <- sum(unpaired_female*mutant_pref_function_matrix*fecundity)/((1/lambda
    )+sum(unpaired_female*mutant_pref_function_matrix*time_matrix))
    gamma_iteration_tab_calc[iteration] <- gamma_iteration



    ## 3D graph of the strategy
    tlm <- 1:tmax
    size <- 1:m
    persp(size, tlm, resident_pref_function_matrix,
        ylab = "tlm", xlab = "size", zlab = "number of unpaired females",
        theta = 160, phi = 10, r = sqrt(3), d = 5,
        col = "black",
        border = "red",
        ticktype = "detailed"
        )


    ###
    ### for the loop
    max_diff_strat <- max(abs(mutant_pref_function_matrix-resident_pref_function_matrix))

    iteration <- iteration + 1
}
```

Appendix 3:

R code for the model presented in Chapter 3

```r
#---------------------------------------------------------
#
# Appendix 3
# R script for the size-assortative mating model, manuscript 2
# effect of sex ratio on size-assortative mating
# carefull, this is a long lasting simulation (several days)
#
#---------------------------------------------------------

rm(list=ls())

# male choice, yes if =1, no if =0
male_choice <- 1
max_time <- 1000
repetition <- 500

## population parameters
growth_rate <- 1.1
male_death_rate <- 0.012
female_death_rate <- 0.012
total_pop_size <- 1000

## sex-ratio = nb males/total_pop_size
sex_ratio <- c(seq(0.1,0.4,0.02),seq(0.405,0.6,0.005),seq(0.62,0.9,0.02))

## vectors for data
pearson_sr <- numeric(length(sex_ratio))
IC95_max_sr <- numeric(length(sex_ratio))
IC95_min_sr <- numeric(length(sex_ratio))


###
### loop to test the effect of sex-ratio on homogamy
###
sr_loop <- 0
cpt <- 0  # count(number of code loop)

for (sr_loop in sex_ratio){

    cpt <- cpt + 1

    ## number of males and females
    nm <- round(sr_loop * total_pop_size)
    nf <- round(total_pop_size - nm)

    ## pearson coefficient of size assortative mating
    coeff_pearson <- numeric(repetition)

    for (iteration in 1:repetition){

        ###############################
        #####     POPULATIONS     #####
        ###############################

        # Females :

        f_no <- c(1:nf)
```

```r
f_size <- round(rnorm(nf,2,0.2),3)
f_max_T <- round((6.75+14.83*f_size),2)
f_T <- round((runif(nf, min=0, max=f_max_T)),2)
## males paired with females
f_no_male <- numeric(nf)
f_size_male <- numeric(nf)
f_T_male <- numeric(nf)

# Males :

m_no <- c(1:nm)
m_size <- round(rnorm(nm,2.75,0.2),3)
m_max_T <- round((6.75+14.83*m_size),2)
m_T <- round((runif(nm, min=0, max=m_max_T)),2)
## females paired with males
m_no_female <- numeric(nm)
m_size_female <- numeric(nm)
m_T_female <- numeric(nm)

################################
##### POPULATION Dynamics  #####
################################

for (time in 1:max_time) {

    #########################
    #      death/birth      #
    #########################

    ### death :

  # individuals randomly die :
    no_male_dead <- m_no[male_death_rate > runif(nm,0,1)]
    no_female_dead <- f_no[female_death_rate > runif(nf,0,1)]

    nb_male_dead <- length(no_male_dead)
    nb_female_dead <- length(no_female_dead)

  # individuals paired with dead individuals become single :
    male_widow <- f_no_male[no_female_dead]
    female_widow <- m_no_female[no_male_dead]

    m_no_female[male_widow] <- 0
    m_size_female[male_widow] <- 0
    m_T_female[male_widow] <- 0
    f_no_male[female_widow] <- 0
    f_size_male[female_widow] <- 0
    f_T_male[female_widow] <- 0

    ### birth :
    # for each death, one birth :

    f_size[no_female_dead] <- round(rnorm(nb_female_dead,2,0.2),3)
    f_max_T[no_female_dead] <- round((6.75 + 14.83 * f_size[no_female_dead]),2)
    f_T[no_female_dead] <- f_max_T[no_female_dead]
    f_no_male[no_female_dead] <- 0
    f_size_male[no_female_dead] <- 0
```

```r
    f_T_male[no_female_dead] <- 0

    m_size[no_male_dead] <- round(rnorm(nb_male_dead,2.75,0.2),3)
    m_max_T[no_male_dead] <- round((6.75 + 14.83 * m_size[no_male_dead]),2)
    m_T[no_male_dead] <- m_max_T[no_male_dead]
    m_no_female[no_male_dead] <- 0
    m_size_female[no_male_dead] <- 0
    m_T_female[no_male_dead] <- 0


    ################################
    ########    AGEING    ########
    ################################

    # every individual comes one day closer to moult :
    f_T <- f_T - 1
    m_T <- m_T - 1

    # paired individuals also come one day closer to moult :
    f_T_male[f_no_male != 0] <- f_T_male[f_no_male != 0] - 1
    m_T_female[m_no_female != 0] <- m_T_female[m_no_female != 0] - 1

    # does anybody moult today (female and male) ?
    nb_moult_today <- length(f_no[f_T <= 0]) + length(m_no[m_T <= 0])


    ############
    ### MOULT ###
    ############

    # if one member of the pair moults, the pair split up :
    if (nb_moult_today > 0) {
        # which female moult today ?
        female_moulting <- f_no[f_T <= 0]
        # among them, which are paired ?
        female_moulting_paired <-f_no[(f_T <= 0) & (f_no_male != 0)]
        # males paired with a moulting female :
        male_paired <- f_no_male[female_moulting_paired]

        # same for males :
        male_moulting <- m_no[m_T <= 0]
        male_moulting_paired <- m_no[(m_T <= 0) & (m_no_female != 0)]
        female_paired <- m_no_female[male_moulting_paired]

        # females become unpaired :
        f_no_male[female_moulting_paired] <- 0
        f_size_male[female_moulting_paired] <- 0
        f_T_male[female_moulting_paired] <- 0
        m_no_female[male_paired] <- 0
        m_size_female[male_paired] <- 0
        m_T_female[male_paired] <- 0

        # males become unpaired :
        m_no_female[male_moulting_paired] <- 0
        m_size_female[male_moulting_paired] <- 0
        m_T_female[male_moulting_paired] <- 0
        f_no_male[female_paired] <- 0
        f_size_male[female_paired] <- 0
        f_T_male[female_paired] <- 0
```

```r
        # individuals grow after moult (+10%) :
        f_size[female_moulting] <- round(f_size[female_moulting]*growth_rate ,3)
        m_size[male_moulting] <- round(m_size[male_moulting]*growth_rate ,3)

        # they begin a new moulting cycle, they are at the maximum time left to
        moult :
        f_max_T[female_moulting] <- round((6.75+14.83*f_size[female_moulting]),2)
        f_T[female_moulting] <- f_max_T[female_moulting]
        m_max_T[male_moulting] <- round((6.75+14.83*m_size[male_moulting]),2)
        m_T[male_moulting] <- m_max_T[male_moulting]
}

###############################
######### PAIRING ##########
###############################

# available females (who and how many ?)
no_female_available <- f_no[f_no_male == 0]
nb_female_available <- length(f_no[f_no_male == 0])

# available males (who and how many ?)
no_male_available <- m_no[m_no_female == 0]
nb_male_available <- length(m_no[m_no_female == 0])

# if some individuals are available :
if ((nb_female_available > 0) & (nb_male_available > 0)){

    # SHAKER : mixing male population
    if (nb_male_available > 1){
        no_male_available <- sample(no_male_available, nb_male_available)
    }

    # we ask each available male in turn :
    for (line in no_male_available){
        no_female_available <- f_no[(f_no_male == 0)]
        nb_female_available <- length(no_female_available)

        if(nb_female_available >=1) {

            ###################
            ### MALE CHOICE ###
            ###################

            # Which females are suitable and available for him ?
            # male choice for females that moult before he does :
            if (male_choice == 1){
                no_female_available <- f_no[(f_no_male == 0) & (f_T < m_T[line])]

            # random choice :
            }else if(male_choice == 0){
                no_female_available <- f_no[f_no_male == 0]
            }

            # How many females are available for this male ?
            nb_female_available <- length(no_female_available)
```

```r
                        # at least one female is available:
                        if (nb_female_available != 0){
                            if (nb_female_available == 1) {
                                no_female_selected <- no_female_available
                            } else if (nb_female_available > 1) {
                              # male choose a female randomly among available and suitable
                              females :
                                no_female_selected <- sample(no_female_available,1)
                            }
                            # we assign the chosen one to the male :
                            m_no_female[line] <- no_female_selected
                            f_no_male[no_female_selected] <- m_no[line]

                            m_size_female[line] <- f_size[no_female_selected]
                            f_size_male[no_female_selected] <- m_size[line]

                            m_T_female[line] <- f_T[no_female_selected]
                            f_T_male[no_female_selected] <- m_T[line]
                        }
                    }
                }
            }

        }

        ### calculation of pearson coefficient at t = max_time
        test_pearson <- cor.test(f_size_male[f_no_male != 0], f_size[f_no_male != 0], method
        = "pearson")
        coeff_pearson[iteration] <- test_pearson$estimate

    }

    ## mean pearson for a given sex_ratio and its CI
    pearson_sr[cpt] <- mean(coeff_pearson)
    IC95_min_sr[cpt] <- mean(coeff_pearson) - 1.96*sd(coeff_pearson)/sqrt(repetition)
    IC95_max_sr[cpt] <- mean(coeff_pearson) + 1.96*sd(coeff_pearson)/sqrt(repetition)
}
```

Appendix 4:

R code for the model presented in Box.1

```r
##--------------------------------
##
## Appendix 4
## R code used in Box.1
## proportion of accepted females
## under the female-sooner norm
##
##--------------------------------


## Simulation:
## loop for the calculation
## of the mean proportion of accepted females


nloop <- 1000
previously_unpaired <- numeric(nloop)
previously_paired <- numeric(nloop)
popsize <- 200

for(i in 1:nloop){
    ## female population
    nf <- popsize/2
    f_size <- round(rnorm(nf,2,0.2),3)
    f_Mmax <- round((6.75+14.83*f_size),2)
    f_M <- round((runif(nf, min=0, max=f_Mmax)),2)

    ## male population
    nm <- nf
    m_size <- round(rnorm(nm,2.75,0.2),3)
    m_Mmax <- round((6.75+14.83*m_size),2)
    m_M <- round((runif(nm, min=0, max=m_Mmax)),2)

    ## calculated proportion of accepted females when
    ## individuals are considered as previously unpaired
    previously_unpaired[i] <- sum((m_M - f_M) > 0)/nf

    ## calculated proportion of accepted females when
    ## individuals are considered as previously paired
    f_M_paired <- f_M[(m_M - f_M) > 0]
    m_M_paired <- m_M[(m_M - f_M) > 0]
    previously_paired[i] <- sum((sample(m_M_paired) - sample(f_M_paired)) > 0)/length(
    m_M_paired)
}

prop_unpaired <- c(mean(previously_unpaired), quantile(previously_unpaired, c(0.025, 0.975)))
prop_paired <- c(mean(previously_paired), quantile(previously_paired, c(0.025, 0.975)))


## real data:
## from Dick & Elwood 1989 and manuscript 1
## bootstrap function for the calculation of
## a confidence interval

boot_data <- function(nb_accepted, nb_rejected){
    nboot <- 1000
    prop_accepted <- numeric(nboot)
```

```r
    for(i in 1:nboot){
        experiment <- c(rep(1, nb_accepted), rep(0, nb_rejected))
        sample_size <- length(experiment)
        boot_x <- sample(experiment, sample_size, replace = T)
        prop_accepted[i] <- length(boot_x[boot_x == 1])/sample_size
    }
    mean_accepted <- c(mean(prop_accepted), quantile(prop_accepted, c(0.025, 0.975)))
    mean_accepted
}


## Dick & Elwood 1989
number_of_accepted <- 42
number_of_rejected <- 8
boot_data(number_of_accepted, number_of_rejected)


## manuscript 1
number_of_accepted <- 112
number_of_rejected <- 10
boot_data(number_of_accepted, number_of_rejected)
```

Communication and teaching

Accepted articles in peer reviewed journals:

Galipaud, M., Dechaume-Moncharmont, F-X., Oughadou, A. and Bollache, L. 2011. Does foreplay matter? *Gammarus pulex* females may benefit from long-lasting precopulatory mate guarding. *Biology letters*. 7, 333-335.

Galipaud, M., Gauthey, Z. and Bollache, L. 2011. Pairing success and sperm reserve of male *Gammarus pulex* infected by *Cyathocephalus truncatus* (Cestoda: Spathebothriidea). *Parasitology*. 138, 1429-1435.

Galipaud, M., Bollache, L. and Dechaume-Moncharmont, F-X. *In press*. Assortative mating by size without a size-based preference: the female-sooner norm as a mate-guarding criterion. *Animal behaviour*.

Articles in preparation:

Galipaud, M., Bollache, L., Oughadou, A. and Dechaume-Moncharmont, F-X. Simple decision rule for mate-switching during precopula in an amphipod crustacean. *In prep*

Galipaud, M., Bollache, L., Wattier, R., Dechaume-Moncharmont, F-X. and Lagrue, C. Overestimation of the strength of size-assortative pairing in taxa with cryptic diversity: a case of Simpson's paradox. *In prep*

Oral communication:

12th – 18th August 2012: 14th ISBE conference in Lund (Sweden).
Communication title: "Linking preferences to mating patterns: size-assortative mating without a preference based on size"

17th – 22nd January 2012: Workshop at the Wissenchaftskollege in Berlin: "Toward a unified approach of pair formation".
Communication title: "mating strategies in mate guarding crustaceans"

17th June 2011: "Forum des jeunes chercheurs" in Dijon
Communication title: "Does foreplay matter? *Gammarus pulex* females may benefit from long-lasting precopulatory mate-guarding."

1st – 4th September 2010: Bath conference "Sexual Selection: integrating behaviour and genomics".
Communication title: "Does foreplay matter? *Gammarus pulex* females may benefit from long-lasting precopulatory mate-guarding."

Poster communication:

16th – 20th Mai 2011: colloque de la Société Française pour l'Etude du Comportement Animal (SFECA).
Communication title: "Does foreplay matter? *Gammarus pulex* females may benefit from long-lasting precopulatory mate-guarding."

Teaching:

November – December 2009, 2010, 2011: tutorial classes in behavioural ecology for first year students at the rate of 20 hours per year.

March – May 2010, 2011: practicals in behavioural ecology for third and fourth year students at the rate of 30 hours per year.

April – May 2010, 2011: practicals in general ecology for second year students at the rate of 20 hours per year.

September 2011: 8 hours practicals in population ecology for fourth year students.

October – December 2011: 37 hours tutorial classes in statistics for first year students.

Summary

Because of strong costs associated with each mating event, females are usually not as available for reproduction as males at any given time. Males are therefore often in competition with each other for access to receptive females, hence leading to strong sexual selection on males. One textbook case of such mating system occurs in moulting crustaceans where females can only be fertilized during a short period following their moult. The resulting strong male-male competition for access to females leads to sexual selection on males. This has favoured the evolution male strategies to monopolize females before their period of receptivity. Such precopulatory mate guarding is widespread among many taxa and represents one of the most striking example of males' competitive traits favoured by sexual selection. However, recent investigations have suggested that because males' sexually selected traits often involve opportunity or mortality costs for them, they should become choosy towards females to compensate for these costs. Using a theoretical approach, we showed that males performing long lasting precopulatory mate guarding should choose larger more fecund females. However, under sequential encounter of potential mates, this choosiness was never very strong, hence pondering the potential for males to actually discriminate among females before entering in precopula. We therefore suggested that males should become choosy after pairing first at random with a female. When encountering an unpaired female of relative better quality than their current female, paired males should switch partners. Contrary to our expectations, even under such simultaneous encounter of two females, males did not seem to assess their relative quality. Instead they decided to change partner when their own female was of low absolute quality. This led to several cases where males actually forewent the possibility of increasing their fitness by pairing with a female of relative better quality. Further investigations are needed to understand the adaptive significance of using only a subset of information in decision making.

These two cases highlight the difficulty of inferring mating pattern from mating preferences only. In the first case, male preference was constraints by competition for access to females while in the second one, sampling processes led to apparent suboptimal mate choices. These potential constraints on decision making have rarely been acknowledge in precopulatory mate guarding crustaceans in spite of their major importance when inferring the causes of a well-known pairing pattern occurring in these species: size-assortative pairing. Size assortment among pairs has mainly been considered to come from a male directional

preference for larger females associated with a large male advantage in getting access to preferred females. However, this hypothesis has received contrasted empirical support and little is known about the underlying pairing process potentially causing size-assortative pairing. As a non-exclusive alternative to this hypothesis, we investigated theoretically the possibility that a state-dependent male mating preference could account for size-assortative pairing. When males chose females which were exclusively closer to moult than them, assortative pairing by size arose under strong male-male competition. Because several preferences can account for a given pattern, this results emphasises the importance of considering the whole pairing process when studying the link between preferences and mate choice.

Besides male mate choice, female strategies may also be of great importance during the pairing process. Contrary to males, females have been suggested to prefer short duration of precopulatory mate guarding because they are supposed to suffer great costs while being paired. Such a sexual conflict over guarding duration may lead to further competition among males and selection pressure to overcome female resistance to pairing. Consequently, this sexual conflict has a major effect on co-evolutionary dynamics between males and females traits. Proving its occurrence is yet challenging because empirical studies often lack of full economical surveys of costs and benefits for females associated with male traits. Females benefits associated with long lasting precopulatory mate guarding have particularly been overlooked in previous studies. Here, we proposed several potential benefits for females and discuss their influence on sexual conflict over guarding duration.