Fake news? The impact of cue mismatch in mating behaviour

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Abstract

Although the role of multiple cues in mate choice have been widely studied, the consequences thereof for receivers remain poorly understood, especially when there is a mismatch between cues. We address this using the spider mite Tetranychus urticae, in which virgin females are highly valuable mates compared to mated females, given first male sperm precedence. We tested how the presence of females of different mating status, and of cues they left in the substrate affected mating behaviour as well as male costs. Male mating attempts were solely affected by substrate cues, being more frequent on patches with cues of virgins, while female acceptance and number of mating events were independently affected by both female identity and substrate cues, being higher when cues stemmed from virgins. Once copulation started, its duration depended mainly on the mating status of the female being fertilized, with the overall amount of time spent mating being higher in matings with virgins than in those with mated females. Male survival costs mirrored their investment in mating, with patches with a mismatch between cues showing intermediate survival costs. The substrate cues left by females are thus instrumental for males to find their mates, but they can also lead to males paying a high survival cost while not reaping the benefit of mating effectively, which suggest they are less reliable but more efficient than cues on females. The benefit of using redundant cues will then hinge upon the frequency of mismatch between cues, which itself should vary with the dynamics of populations.

Fake news? The impact of cue mismatch in mating behaviour

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Data Availability Statement

Analyses reported in this article can be reproduced using the data and code that will be publicly available in Dryad upon acceptance. For reviewing purposes, we provide the corresponding private link: https://datadryad.org/stash/share/OCdqzNAQDww5T9e8jtUIUiWmTyptqxkcOAf6nQtc8UY

Abstract

Although the role of multiple cues in mate choice have been widely studied, the consequences thereof for receivers remain poorly understood, especially when there is a mismatch between cues. We address this using the spider mite *Tetranychus urticae* , in which virgin females are highly valuable mates compared to mated females, given first male sperm precedence. We tested how the presence of females of different mating status, and of cues they left in the substrate affected mating behaviour as well as male costs. Male mating attempts were solely affected by substrate cues, being more frequent on patches with cues of virgins, while female acceptance and number of mating events were independently affected by both female identity and substrate cues, being higher when cues stemmed from virgins. Once copulation started, its duration depended mainly on the mating status of the female being fertilized, with the overall amount of time spent mating being higher in matings with virgins than in those with mated females. Male survival costs mirrored their investment in mating, with patches with a mismatch between cues showing intermediate survival costs. The substrate cues left by females are thus instrumental for males to find their mates, but they can also lead to males paying a high survival cost while not reaping the benefit of mating effectively, which suggest they are less reliable but more efficient than cues on females. The benefit of using redundant cues will then hinge upon the frequency of mismatch between cues, which itself should vary with the dynamics of populations.

Keywords: mate choice; mating costs; multiple cues; reproductive behaviour; spider mites; first male sperm precedence

Introduction

In many taxa, individuals use multiple cues to search for mates and engage in copulations . Along the years, several hypotheses have been put forward to explain the role of different cues in mate decision (see detailed classifications in : a) each cue may convey information about different mate qualities, the cues together increasing the accuracy of assessment, b) cues can be redundant but together improve discrimination, reducing errors associated to each cue, and/or c) cues can work differently in distinct environments and/or at dissimilar distances.

Recent work has been consolidating the importance of studying the role of multiple cues in varying and complex environments , under the assumption that individuals often experience fluctuations in their environment, such as changes in resource availability or in the density, quality and sex ratio of conspecific and heterospecific individuals. In such varying and complex environments, relying on multiple cues may contribute to an accurate and fast response by the receiver across environmental conditions . However, a disruption in the information transfer between signallers and receivers, via excess noise or a mismatch between cues, is also more likely in complex environments .

Mismatches among cues can occur when cues have different susceptibilities to changes in the environment, or when they persist for different periods of time . For example, mate quality can be perceived simultaneously via ephemeral cues, like many behavioural traits, and more permanent cues such as some morphological traits, with the former functioning as indicators of current condition thus changing with the environment, and the latter indicating lifetime performance and remaining virtually unchanged . That is the case of the field cricket (*Gryllus campestris*) that uses both body size and chirp rate as cues for mate choice, typically giving priority to body size, the most permanent cue . In variable environments, however, fixed cues may become unreliable indicators of male quality , in which case the use of ephemeral cues may be favoured. A similar phenomenon is observed in the*Pieris rapae.* During development, this butterfly relies on both temperature and photoperiod to predict conditions at adult emergence. Climate warming promotes the mismatch between these two cues, affecting temperature but not the photoperiod, which can result in a sub-optimal wing melanisation phenotype upon emergence, provided both cues continue to be used . The mismatches between cues can also occur because of sexual conflicts , with signallers emitting dishonest signals owning to diverging interests between sexes . Under all these scenarios, perceiving more than one cue can increase, rather than reduce, uncertainty about which decision to make.

While the costs of emitting multiple cues are extensively described , the costs for receivers are less well studied, especially when considering mismatches between cues. Besides the expected costs associated with increased energetic and cognitive effort needed to process more than one cue , costs for receivers are likely to be associated with missing opportunities of mating with a suitable mate or with attempting to mate with unsuitable mates . For instance, cues used to assess conspecific mate quality sometimes overlap with those used for species recognition, or kin recognition . This is the case in *Gryllus integer* males for which the stimuli of heterospecific females overrides conspecific chemical cues during mating trials . To avoid losing suitable mates, one possibility is to evolve a lower mate acceptance threshold in environments with discordant cues , conceivably at the expense of accepting less desired mates . The same reduction in the acceptance threshold can be observed when the costs of assessing multiple cues are higher than the benefits of using them to only mate with suitable mates, in which case individuals can ignore the least reliable cues and accept a certain level of uncertainty . The opposite is expected if the goal is to prevent matings conferring little to no reproductive success. Therefore, the optimal use of cues and corresponding behaviour should depend on the balance between the costs of acceptance and rejection errors .

So far, the studies exploring the response of individuals to mismatches between cues in the context of mate choice have largely disregarded cases in which males are receivers and females are signallers of cues. Addressing this issue can be particularly relevant in species with first male sperm precedence, where female mating status discrimination is essential for male mating success. Indeed, under this pattern of sperm precedence, mating with mated females provides low, if any, fertilization opportunities, whereas mating with virgin females strongly contributes to reproductive success . Accordingly, it has been shown that males of these species have evolved the ability to discriminate female mating status and modulate their reproductive behaviour based on the cues presented by females . Yet, the behaviour of males when cues provide discordant information concerning the female mating status, as well as the associated costs thereof, remain largely unknown.

To fill this gap, we observed the mating behaviour of male and female two-spotted spider mites (*Tetranychus urticae)* in environments with information concerning the female mating status coming from two sources, the female itself and the cues it leaves in the substrate. Spider mites have first male sperm precedence and, accordingly, males prefer to mate with virgins, basing their decision upon volatile and substrate cues . Furthermore, matings with virgin females take less time to start, are longer, and induce more survival costs in males than matings with mated females . All this suggests that male reproductive investment in matings with virgins and with mated females is not the same. However, matings involving mated females are frequently observed in laboratory populations , despite often leading to lower fecundity . This suggests that discrimination in this species is not perfect and may depend on the composition of cues present in the environment. Spider mite populations occur in variable environments, as they colonize seasonal resources such as agricultural crops . Moreover, they disperse among patches after a variable number of generations in the same patch, following a subdivided haystack population structure . This results in a scenario of cyclic waves of virgin and mated females across time within the same plant, which fosters the conditions for a mismatch between the different cues signalling female mating status. Here, we tested the consequences of cue mismatch within this context.

Materials and Methods

Spider mite populations and rearing conditions

The spider mite population used was created from an outbred population of *Tetranychus urticae* , established in 2016 at the host laboratory, by merging six populations collected in the field around Lisbon in 2013 . The population was reared in large numbers (>200) on bean plants (*Phaseolus vulgaris* , Fabaceae, var. Contender; Germisem Sementes Lda, Oliveira do Hospital, Portugal), under controlled conditions (25°C, photoperiod of 16L: 8D). All bean plants used in the experiment were grown for 14 days in an herbivore-free climatic chamber under the same controlled conditions as spider mites.

Experimental Setup

All experiments were done on bean leaves. Females and males were isolated from the base population on detached leaves at the quiescent stage, immediately before completing the last moult. This way, all individuals used in the experiment shared the same age-at-maturity, and virginity was ensured in both sexes before they were allocated to different treatments.

To create different environments for males, multiple groups of 10 virgin females were randomly assigned to patches (leaf discs of 2.55 cm^2) with 3 virgin males. Behaviour was observed for 1 hour and, when matings occurred, females were transferred in groups of 10 to a new empty patch of the same size. Simultaneously, groups of 10 virgin females were directly transferred to similar empty patches without ever being in contact with males. Both types of females were left on those patches during 24h such that they could release cues that remained on the substrate. Those females were then removed, and 5 new females (either mated or virgin) were placed on those patches. Subsequently, one focal male was added to all patches. Previous work done on similar conditions shows that male spider mites use volatile and substrate cues to choose between virgin and mated females . Thus, with our setup, we created the conditions for mate discrimination to take place in an environment in which there were matching female cues (e.g., virgin females emitting cues on patches previously impregnated with cues released by virgin females) or in which there was a mismatch between the cues emitted by the females present on the patch and the cues that were left on the substrate by previous females (e.g., virgin females emitting cues on patches previously impregnated with cues released by mated females). Henceforth, for simplicity, we refer to the cues left on the patch by virgins or by mated females that were removed prior to the beginning of the mating sessions as "substrate cues" and the cues emitted by females present on the patch (including their own behaviour) as "female mating status".

Male and female behaviour, i.e., the number of male mating attempts, the frequency of female acceptance, the number of mating events and copulation duration were observed for 1 hour. A mating attempt was registered whenever a male touched the female with the two front legs and started bending its opisthosoma . Whenever a mating attempt resulted in the insertion of the male aedeagus into the female abdomen for more than one minute, the observer registered it as the occurrence of a mating event . The frequency of female acceptance was calculated as the number of mating events over the number of mating attempts. Copulation duration was registered as the time in seconds a male spent with his aedeagus inside a female. Note that females were not removed from the patch during a mating session, so mating events could have occurred with mated females in patches with virgin females at later stages of the mating session.

Subsequently, males were transferred individually to a new patch (2.55 cm^2), made from uninfested bean plants, and their survival was followed daily, to measure whether different mating histories would translate into a longevity cost. Death was classified as natural (i.e., the corpse was found on the patch) or censored (i.e., males died by drowning or by being accidentally stuck in the leaf or squeezed).

This experiment was carried out in 21 mating sessions divided in 8 days, and in total, 84 males and 420 females, corresponding to 21 males and 105 females per treatment (i.e., combination of type of substrate cues and female mating status) were observed.

Statistical analyses

All analyses were carried out using the R statistical package (v. 3.5.2). The same model structure was followed for the analysis of all traits: the substrate cues (i.e., cues left on the patch by virgins or by mated females that were removed prior to the beginning of the mating sessions) and the female mating status (i.e., virgin or mated females present on the patch during the mating session) were fitted as fixed explanatory variables, whereas block (the day and time of the day at which the experiment was done) was fitted as a random explanatory variable (see Table S1).

Copulation duration was examined as "copulation duration of the first mating only" and "copulation duration across mating events". In the analysis of the latter variable, the order of each copulation (i.e., whether it was the first, second, third mating, etc) was added as a covariate. All possible interactions between fixed factors were included.

The number of mating attempts and the number of mating events were analysed using a Poisson distribution (*glmer* , lme4 package; . The frequency of female acceptance was analysed using a binomial distribution (*glmer*

, lme4 package), with the formulation of the dependent variable including the number of female rejections and acceptances within a *cbind* function. The duration of the first mating and the copulation duration across events were tested for normality and analysed using linear mixed-effect models (*lmer* , lme4 package; . Male survival was analysed using a Cox proportional hazards mixed-effect model (*coxme* , coxme package; , with the death of males being classified as natural or censored.

All maximal models were simplified by sequentially eliminating non-significant terms from the highest- to the simplest-order interaction . The significance of the explanatory variables was determined using Wald F tests, for continuous distributions and χ^2 tests for discrete distributions .

Results

Results from all statistical analyses are presented in Table S2. Males approached females more often in patches with substrate cues of virgin females, independently of the mating status of the females present on the patch (substrate cues x mating status: $\chi^2{}_1$=1.104, P=0.293; patch cues: $\chi^2{}_1$=54.323, $P<0.01$; mating status: $\chi^2{}_1$= 2.055, P= 0.152; Fig. 1a). Virgins accepted male mating attempts more often than mated females and the frequency of female acceptance was higher in patches with cues of virgin females (patch cues x mating status: $\chi^2{}_1$=2.625, P=0.105; patch cues: $\chi^2{}_1$=8.553, $P<0.01$; mating status: $\chi^2{}_1$= 64.252, P= $P<0.01$; Fig. 1b). The number of mating events was also affected by both the substrate cues and the mating status of the female independently (patch cues x mating status: $\chi^2{}_1$=1.274, P=0.259; patch cues: $\chi^2{}_1$=35.445, $P<0.001$; mating status: $\chi^2{}_1$= 17.89, $P<0.001$; Fig. 1c), with number of mating events being lower when matings were with mated vs virgin females and when they occurred on patches with cues of mated vs virgin females (Fig. 1c).

The first mating of a male lasted longer when it involved virgin females and when it occurred in patches with cues of virgins than when it involved mated females and occurred on patches with cues from mated females (patch cues x mating status: $F_{1,60}$=0.002, P=0.966; patch cues: $F_{1,61}$=4.737, P=0.033; mating status: $F_{1,61}$=70.367, $P<0.001$; Fig. 2a). Copulation duration was always significantly higher in patches with cues of virgins than in patches with cues of mated females (patch cues: $F_{1,72.70}$=4.624, P=0.035; Fig. 2c) but it significantly decreased across mating events and this decrease was steeper when males were placed in patches with virgin females (copulation order x mating status: $F_{1,364.06}$=5.652, P=0.018).

Male survival on patches with cues left by virgin females and the presence of virgin females was reduced compared to that of males on patches with cues left by mated females or with mated females present (patch cues x mating status: $\chi^2{}_1$=0.496, P=0.481; patch cues: $\chi^2{}_1$=4.283, P=0.038; mating status: $\chi^2{}_1$= 8.774, P= 0.003; Fig. 3).

Discussion

Here we examine the impact of multiple cues on the mating behaviour of spider mites. We found that the number of mating attempts was only influenced by cues left on the patch prior to the mating sessions, being higher in patches with cues of virgins. In turn, female acceptance and the number of mating events of the couple were affected by both substrate cues and the mating status of the females, being the highest in patches with virgins and with substrate cues of virgin females and the lowest on patches with mated females and with substrate cues of mated females. Once copulation started, its duration seemed to depend mainly on the mating status of the female being fertilized, with the overall amount of time invested in mating being higher in matings with virgins than with mated females. Ultimately, male survival costs mirrored the reproductive investment of males, with patches in which there was a mismatch between cues showing an intermediate number of mating events, cumulative copulation duration and survival costs.

In species with first male sperm precedence, like spider mites and many spiders, the sperm from the first insemination sires most of the offspring, thus virgins are more valuable mates than mated females. It is thus expected that males exhibit a preference for virgin females. The intensity of male eagerness observed here when males were presented with matching cues is aligned with such expectations and with previous studies done in this and other species with the same pattern of sperm precedence . When exposed to discordant cues, males disregarded the cues emitted by the female present, basing their pre-copulatory mating behaviour solely on the substrate cues on the patch. These cues are obviously less reliable determinants of the status of the female on those patches than cues emanating from the female itself. Although we are unaware of studies investigating the duration of substrate cues, results shown here, together with other studies done on mites (Rodrigues et al. 2017, Magalhães et al. 2005) clearly indicate that cues remain on patches at least 24h after the individuals move (or are removed). The use of more unreliable cues in pre-copulatory mating behaviour might help explain why, in spider mites and perhaps in species with a similar pattern of sperm precedence, matings with mated females are frequently observed, despite their weak reproductive value.

Unlike male eagerness, female acceptance depended both on the substrate cues and on the females' own mating status, being weaker not only in mated females, but also in patches with substrate cues of mated females. That virgins accept more matings than mated females goes in line with the expectations for species with this pattern of sperm precedence and with what is known about spider mites reproduction: first, only virgin females receive genetic benefits by mating ; second, multiple mating does not provide females with any non-genetic benefit and can even lead to reduced fecundity . The effect of the substrate cues on female acceptance might seem more cryptic. Yet, one could speculate that females accept more matings in patches with substrate cues of virgins because those are the patches in which the number of mating attempts is higher and thus, resistance is expected to be more costly. This strategy, called "convenience polyandry", should occur under intense harassment, when by accepting more mates than their optima, females suffer fewer costs than by resisting them . Such is the case for instance in female water striders that modify their mating rate based on the relative costs of mating and of resisting mating attempts .

The combination of male eagerness to mate and the frequency of female acceptance is reflected in the number of mating events observed here. When there was no mismatch between cues, and given the pattern of sperm precedence of this species, the interest of males and females were aligned: in patches with cues of virgins, both sexes are willing to mate, thus the number of mating events was the highest; in patches with cues of mated females, none of the sexes directly benefits by mating , so the number of mating events was the lowest. In the other two treatments where there was a mismatch between cues, because males only used substrate cues but females responded according to their own mating status and to the substrate cues, the response of the two sexes was not aligned, resulting in intermediate number of mating events.

Copulation duration was shorter in matings with mated females than in matings with virgins, which in several species has been suggested to reflect a lower investment by males towards females of lower reproductive value . In the case of spider mites, shorter copulations should correspond to reduced investment in post-copulatory guarding , that is typically used as a strategy to guarantee sperm precedence. This trait seems to be more affected by the mating status of the female mating than by substrate cues present in the environment, with copulation duration across mating events decreasing faster in patches with virgin females, regardless of the substrate cues present. Thus, although the substrate cues left in a patch are important for mate acquisition, they seem to play a less significant role in post-copulatory strategies in spider mites. Evidence of adjustments in the use of cues during an individual lifetime is manyfold . For instance, in the bushcricket, *Ephippiger diurnus* , young males adjust their investment in spermatophore production based on social (acoustic) experience, while old male invest equally across social environments .

The response of males to multiple cues, including both pre- and post-copulatory behaviours, should come at some costs. Previously, it was shown that, in male spider mites, survival is affected differently depending on the mating status of their reproductive partners : matings with virgin females result in high offspring yield but reduced male survival, while matings with mated females lead to no offspring but also fewer survival costs. Again, being exposed to discordant cues influenced this trait. First, we saw that, in patches occupied by mated females, males had lower survival when substrate cues were from virgins than when they were from mated females only. It seems that in these cases, the existence of a mismatch leads to an over-investment in ineffective matings. Still, this behaviour could be maintained not to risk rejecting mating opportunities with suitable females, as proposed by Reeve . In his model, Reeve shows that males are expected to exhibit more permissive mating acceptance thresholds as the value of the desirable female increases and the costs of accepting a wrong female decreases, which are the exact conditions we find in this system. Indeed, virgin females are highly valuable compared to mated females and the costs for males of mating with mated females is quite low . An equivalent decrease of the acceptance threshold would be expected if assessing multiple cues was too costly, in which case one would expect individuals to neglect the least reliable cue , that is the cues left on the substrate by mated females.

Male survival in patches with mismatches between cues is higher than in patches with cues of virgins only. In these patches, the number of mating attempts is similar to that in patches with virgins, but the total number of matings and the total amount of time spent copulating is significantly lower. This suggests that the number of mating events and/or postcopulatory events are important determinants of male mating costs, ensuring a reduction in the costs of reproduction in mating with less valuable females. Moreover, male survival was higher in patches with virgin females but substrate cues of mated females, than in patches with cues of virgins only. Therefore, it seems that in these conditions, males invest less in effective matings, possibly via a reduction in the number of male mating attempts and in the total amount of time spent mating. However, we have not tested whether the observed reduction in copulation duration is translated into reduced mating success and previous results suggest copulation duration does not correlate positively with offspring production .

We did not measure the composition of the cues that males were exposed to, but we can make a few inferences from the patterns observed in male behaviour upon exposure. For example, we do not know whether the cues of the females themselves have the same composition as those left in the substrate. This is however not very likely, as different components of male mating behaviour react differently to the different combinations of mating cues from virgins and/or mated females. Another possibility is that the different treatments result only in a different quantity, rather than quality, of cues. For example, it may be that only virgin females produce cues. Our results are compatible with this possibility. Still, this would mean that males are exposed to situations in which the information stemming from the females themselves and the substrate they occupy are either concordant or discordant.

The optimal use of cues and corresponding behaviour should depend on the balance between the costs of acceptance and rejection errors and this, in turn, should vary with the dynamics of the social and ecological environment. In spider mite populations, individuals disperse among patches after a variable number of generations in the same patch, following a subdivided haystack population structure . Such cycles of colonization-expansion foster the conditions for cue mismatch within a patch. Indeed, while the cues emitted by females will change simultaneously with the shift in mating status, the cues left on the patch should remain unaltered for some time after this shift. While these cues seem to be less reliable than the cues emitted by females themselves, they are probably accessible at a larger scale than those of the female itself, allowing males to move in the direction of areas with suitable mates (i.e., virgins) before their competitors. This should be highly advantageous in species with first male sperm precedence. These findings could thus have important implications for mating system evolution, potentially helping to explain why female multiple mating is maintained in species with first male sperm precedence. Still, the benefit of using multiple, sometimes discordant, cues will hinge upon the frequency of discordance among cues, which itself should vary with the dynamics of populations.

References

Figure 1. Male and female pre-copulatory mating behaviour and the corresponding number of mating events in response to substrate cues and female mating status. a) Number of male mating attempts, b) proportion of mating attempts accepted by females and c) number of mating events. Males were exposed for 1 hour to 5 virgin or mated females in patches impregnated with cues of virgin or mated females. Circles represent individual replicates. Black circles –patches with mated females; grey circles – patches with virgin females; open circles – patches with cues of virgin females; full circles – patches with cues of mated females.

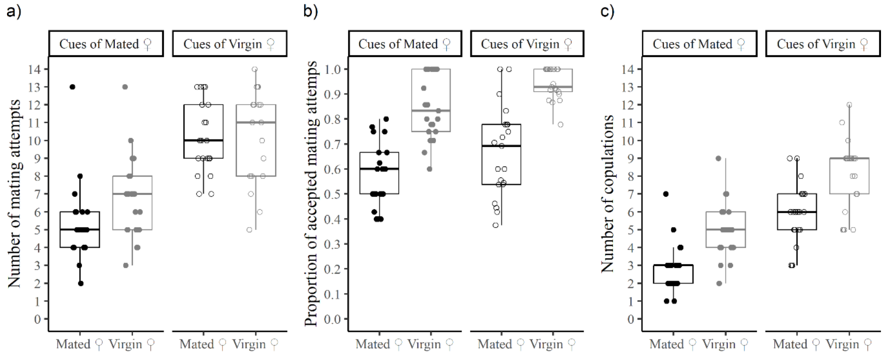


Figure 2. Duration in seconds of a) first matings and b) across mating events, in response to patch cues and female mating status. Males were exposed for 1 hour to 5 virgin or mated females in patches impregnated with cues of virgin or mated females. Circles represent individual replicates. Black circles –patches with mated females; grey circles – patches with virgin females; dashed lines and open circles – patches with cues of virgin females; continuous lines and full circles – patches with cues of mated females.

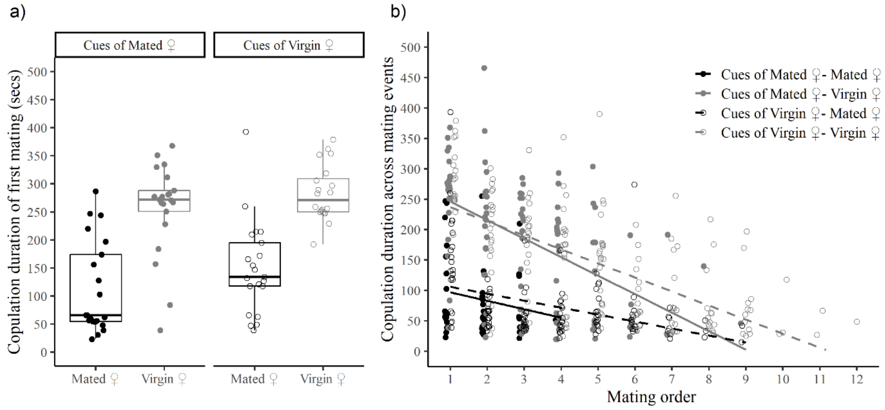
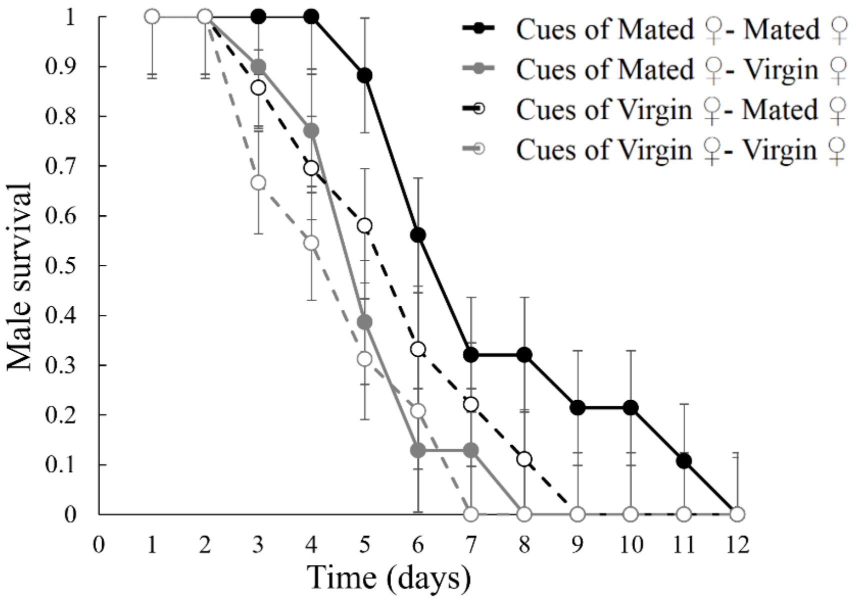


Figure 3. Male survival curves in response to patch cues and female mating status. Male survival was followed daily after males being exposed for 1 hour to 5 virgin or mated females in patches impregnated with cues of virgin or mated females. Circles represent mean values per day per treatment. Black circles –patches with mated females; grey circles – patches with virgin females; dashed lines and open circles – patches with cues of virgin females; continuous lines and full circles – patches with cues of mated females. Vertical bars correspond to standard errors of the mean.



Male survival
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0 1 2 3 4 5 6 7 8 9 10 11 12
Time (days)
Cues of Mated ♀- Mated ♀
Cues of Mated ♀- Virgin ♀
Cues of Virgin ♀- Mated ♀
Cues of Virgin ♀- Virgin ♀