

Fake news? The impact of information mismatch in mating behaviour

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Abstract

Multiple cues are often used for mate choice in complex environments, potentially entailing mismatches between different sources of information. We address the consequences thereof for receivers using the spider mite *Tetranychus urticae*, in which virgin females are highly valuable mates compared to mated females, given first male sperm precedence. Accordingly, males prefer virgins and distinguish them using cues from the females and/or that are present on the substrate. Whereas the former are more reliable, the latter may allow for a faster or more long-distance response. However, there can be mismatched information between cues as females move and/or mate. Here, we tested the consequences thereof by exposing males to mated or virgin females on patches previously impregnated with cues deposited by females of either mating status. Male mating attempts were solely affected by substrate cues while female acceptance and the number of mating events were independently affected by both cues. Copulation duration, in contrast, depended mainly on the mating status of the female, with the number of copulations and the total time spent mating being intermediate in environments with mismatched information. Ultimately, male survival costs mirrored male investment in mating. These results suggest that, in environments with mismatched information, the substrate cues left by females are instrumental for males to find their mates, but they can also lead to males paying survival costs without the associated benefit of mating effectively, or suffering reduced costs at the expense of losing effective mating opportunities. The benefit of using multiple cues will then hinge upon the frequency of information mismatch, which itself should vary with the dynamics of populations.

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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

Multiple cues are often used for mate choice in complex environments, potentially entailing mismatches between different sources of information. We address the consequences thereof for receivers using the spider mite *Tetranychus urticae*, in which virgin females are highly valuable mates compared to mated females, given first male sperm precedence. Accordingly, males prefer virgins and distinguish them using cues from the females and/or that are present on the substrate. Whereas the former are more reliable, the latter may allow for a faster or more long-distance response. However, there can be mismatched information between cues as females move and/or mate. Here, we tested the consequences thereof by exposing males to mated or virgin females on patches previously impregnated with cues deposited by females of either mating status. Male mating attempts were solely affected by substrate cues while female acceptance and the number of mating events were independently affected by both cues. Copulation duration, in contrast, depended mainly on the mating status of the female, with the number of copulations and the total time spent mating being intermediate in environments with mismatched information. Ultimately, male survival costs mirrored male investment in mating. These results suggest that, in environments with mismatched information, the substrate cues left by females are instrumental for males to find their mates, but they can also lead to males paying survival costs without the associated benefit of mating effectively, or suffering reduced costs at the expense of losing effective mating opportunities. The benefit of using multiple cues will then hinge upon the frequency of information mismatch, which itself should vary with the dynamics of populations.

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

Introduction

In many taxa, individuals use multiple sources of information 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. The use of multiple cues is particularly useful in varying and complex environments, as information transfer to receivers may be disrupted in such environments due to excess noise and/or a mismatch between cues.

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 instance, during development, the butterfly *Pieris rapae* relies on both temperature and the photoperiod to evaluate climatic 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. Likewise, mate quality can be perceived via both ephemeral cues (e.g., behavioural traits), and more permanent cues such as morphological traits. That is the case of the field cricket (*Gryllus campestris*) that uses both body size and chirp rate in males as indicators of mate quality, 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.

Whereas the use of multiple cues can be beneficial in many contexts, their use also come at some costs. First, processing information stemming from multiple cues is expected to lead to increased energetic and cognitive investment. Second, when there is mismatched information, using multiple cues may lead to an inaccurate response from the receiver. When this perception of cues concerns mate choice, costs for receivers are likely to be associated with missing opportunities of mating with a suitable mate or with investing in matings with

unsuitable mates . For example, in *Gryllus integer* males the stimuli of heterospecific females compromise conspecific chemical cues detection during mating trials, leading to equal intensity of heterospecific and conspecific courting . Therefore, the optimal use of multiple cues and corresponding mating behaviour should depend on the balance between the costs of acceptance and rejection errors .

The existence of cues mismatch in mate choice can be particularly disadvantageous 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 the ability to discriminate female mating status, preferring the virgins, and modulate their reproductive behaviour based on the cues presented by females . Yet, the behaviour of males when discordant information concerning the female mating status is provided, 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 volatiles released by females into the air and/or substrate cues remaining on the patches . 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 temporal mismatch between the different cues signalling female mating status. Here, we tested the consequences of information mismatch within this context. We predict that, in an environment with information mismatches, the chance of mating with less favourable females and losing valuable mating opportunities is higher than in environments with concordant information, which should influence the overall mating costs suffered by males.

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.

Previous work shows that to choose between virgin and mated females male spider mites use volatiles emitted by females and substrate cues left by females on patches . Based on this information, we created with our setup the conditions for mate discrimination to take place in an environment in which there was matching information (e.g., virgin females on patches previously impregnated with cues released by virgin females)

or in which there was a mismatch between the information released by the females present on the patch and the cues that were left on the substrate by previous females (e.g., virgin females on patches previously impregnated with cues released by mated females). To create these different environments for males, multiple groups of 10 virgin females were randomly assigned to patches (leaf discs of 2.55 cm²) 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. 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 information emitted by females present on the patch (including their own behaviour) as “female mating status”. Note that, although we are aware that chemical information is sufficient for mate choice (Rodrigues et al. 2017), it is possible that other cues or signals (e.g., tactile, visual) are used by males as well (Royalty et al. 1993). In any case, we assume that the information provided by the presence of a female is composed of multiple cues that are concordant, and thus, for simplicity, consider “female mating status” a unit of information. Likewise, substrate cues include web, faeces, eggs and any chemical information left behind by females; once more we consider those a unit of information. We cannot rule out the possibility that new substrate cues are being deposited by the females present in the patch, but given the short duration of trials, these are not expected to play a significant role in the behaviours described.

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. It has been shown that females can reject male mating attempts by moving away from them (Rodrigues et al. 2020). As such, we also studied the frequency of female acceptance, 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 cm²), 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. This model included the number of matings as a covariate.

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: $\chi^2_1=64.252$, $P<0.01$; 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). Indeed, the number of mating events was 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). The number of matings did not influence male survival ($\chi^2_1=0.051$, $P=0.821$).

Discussion

Here we examine the impact of multiple cues and the potential resulting information mismatch 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 was 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. As a consequence, the number of mating events was affected by both substrate cues and the mating status of the females, such that the number of copulations was intermediate in environments with mismatched information. 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 and intermediate in environments with mismatched information. Ultimately, male survival mirrored the reproductive investment of males,

with patches in which there was mismatched information showing intermediate 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. Accordingly, males exhibit a preference for virgin females, in this and other species with the same pattern of sperm precedence. Such result is recapitulated here in patches with concordant cues, with mating eagerness, copulation duration and survival costs being the highest in patches with virgins.

When exposed to discordant information males based 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 information emanating from the female itself. Thus, the fact that pre-copulatory mating behaviour is triggered by substrate cues only suggests that these cues are the most far-reaching of the two, being beneficial in mate searching. A non-exclusive alternative is that this less accurate cue is cheaper to assess, in which case it may pay off to consider it alone (Fawcett and Johnstone 2003). In the field cricket (*Gryllus integer*), calling songs are used as long-distance cues to find mates while short-range chemical cues are employed to assess mate quality (Lenard & Hedrick 2010). Interestingly, being exposed to attractive long-distance cues results in a quicker positive response to short-distance cues, which may suggest a reduced investment in the assessment of the latter cue in this species (Lenard & Hedrick 2010). A similar pattern could be taking place here. The use of more unreliable information 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, with increased acceptance in mated females and reduced acceptance in virgins in environments with mismatched information, compared to concordant environments. Possibly mated females accept more matings in patches with substrate cues of virgins because those are the patches in which the number of male mating attempts is the highest 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. In turn, reduced acceptance of virgin females in patches with substrate cues of mated females could be a byproduct of male's reduced eagerness to mate in those patches, that may result in meek, and thus easy to reject, mating attempts. Importantly, because females' pre-copulatory behaviour depended both on their own mating status and on the substrate cues present but that of males only relied on substrate cues, the response of the two sexes was not aligned, resulting in intermediate number of mating events in environments with mismatched information.

Once copulation started, the response of males seemed to be more affected by the mating status of the female mating than by substrate cues present in the environment. This is probably because, once males reach the females, they have access to the more reliable cues emanating from the female itself, and can thus use those to accurately modulate their post-copulatory strategies. Still, substrate cues played a, albeit less significant, role in copulation duration. Indeed, when exposed to virgin cues, males mated for a longer period with both virgin and mated females than when exposed to cues of mated females. This suggests the information obtained before mating keeps influencing mating behaviour even when more accurate cues are available.

The response of males to multiple cues, including both pre- and post-copulatory behaviours, must 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. 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 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 chemical composition of the different sources of information that males were exposed to, nor do we know exactly which cues are used by males to choose between virgin and mated females. Indeed, we know that substrate cues and volatiles emitted by females are sufficient for male choice (Rodrigues et al. 2017) but other cues, such as visual and tactile cues, not being necessary, may play a role (Royalty et al. 1993). In addition, substrate cues themselves, considered here as a unit of information, include web, faeces, eggs and any chemical compounds deposited by females in the patches. Web is used, among other functions, in mate searching behaviour (Penman and Cone 1972, 1974) but the role of other substances is unknown. Still, we can make a few inferences from the patterns observed in male behaviour upon exposure. For example, we do not know whether the chemicals used by males to assess females themselves are the same present on the substrate. This is however not very likely, as different components of male mating behaviour react differently to the different combinations of cues from virgins and/or mated females. Another possibility is that only virgin females produce cues. Our results are compatible with this possibility. Regardless, this would still 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 information 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, which set the stage for different selection pressures to operate upon mating cues and their perception (Alpedrinha et al. 2019). 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 information mismatches within a patch. Indeed, while the information 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 information provided by females themselves, they are probably accessible at a larger scale, 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 them, 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.

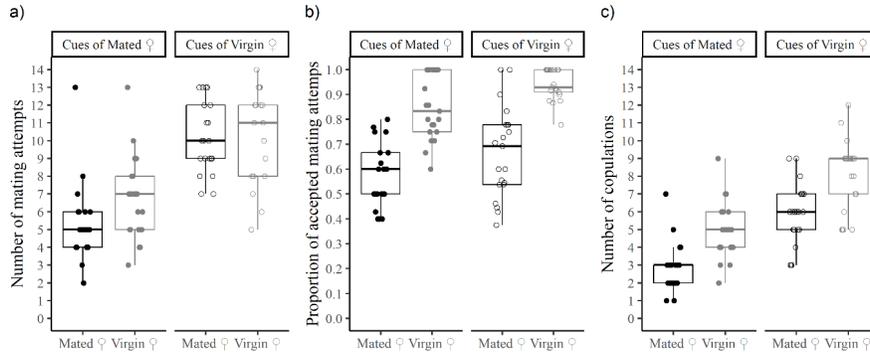


Figure 2. Duration in seconds of a) first matings and b) across mating events, in response to patch cues and female mating status. Males were exposed for 1 hour to 5 virgin or mated females in patches impregnated with cues of virgin or mated females. Circles represent individual replicates. Black circles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

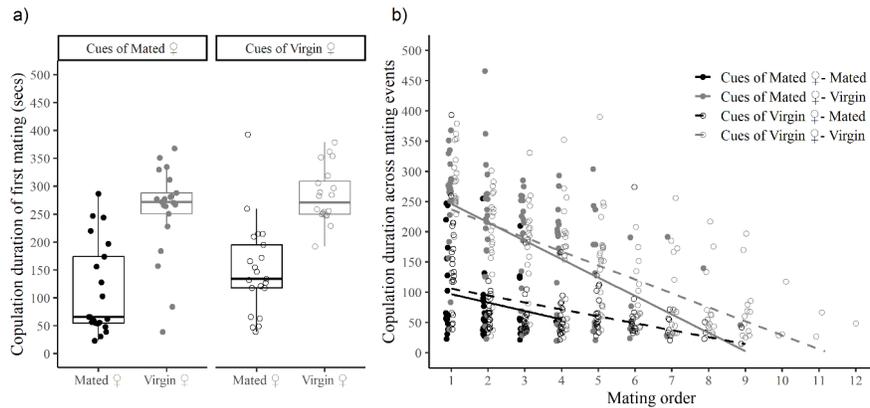


Figure 3. Male survival curves in response to patch cues and female mating status. Male survival was followed daily after males being exposed for 1 hour to 5 virgin or mated females in patches impregnated with cues of virgin or mated females. Circles represent mean values per day per treatment. Black 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.

