



# Females' sequential mating decisions depend on both the quality of the courting male and the quality of the potential mates in a blood-sucking bug

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## Abstract

This study investigates whether female sequential decisions on accepting or rejecting a courting male are based exclusively on the quality of the male present or are affected by the quality of potential mates. We used blood-sucking bugs *Rhodnius prolixus* as experimental subjects as females' exhibit stereotyped discrimination behavior towards undesirable males, which allows sequential testing of a female's preference for each male. We expect higher levels of female rejection when the benefit of finding a better quality male overrides the cost of rejecting a mating opportunity. Male quality was manipulated through changes in the feeding regime prior to the experiment because in this species nutritional condition affects males' reproductive success. Females were simultaneously exposed to both a focal male currently available and a background male potentially available. The quality of each focal and background male could be either low or high and all the possible combinations were tested for each pair (low/high, high/low, high/high, low/low). We found that female rejection behavior depends not only on the quality of the focal male but also on the quality of the background male. This behavioral flexibility may allow females to maximize the benefits of their mating decisions.

## Significance statement

Using an experimental approach, we study how females of a blood-sucking bug assess male quality and decide whether to accept or reject a mating opportunity in a sequential choice paradigm. Male quality (high/low) and male availability (current/potential) were manipulated. We show that female rejection depends on both the quality of the current and potential mates. Female rejection increases when better quality males are potentially available. This behavioral flexibility may allow females to maximize the benefits of mating decisions.

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Gabriel Manrique and Lorena Pompilio contributed equally to this work.

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## Introduction

Females can encounter a number of potential mates, either sequentially or simultaneously, before making a mating decision. There is substantial empirical evidence focusing on the traits, cues, and sampling tactics used by females to assess male quality and choose a mate under simultaneous choice situations, where a female is presented with a pair (or a group) of males which differ phenotypically (Janetos 1980; Wittenberger 1983; Bakker and Milinski 1991; Murphy and Gerhardt 2002; Wiegmann et al. 2013). However, with few exceptions, potential mates often encounter each other sequentially under natural conditions (Real 1990, 1991; Gabor

and Halliday 1997). Little is known about the decision rules used by females when they are courted by single males and have to make sequential choices (Schäfer and Uhl 2005). The reason may lay in the fact that female choice is generally subtle. As a consequence, finding a clear and direct measure of female preference under sequential choice scenarios is not easy. Here, we chose blood-sucking bugs *Rhodnius prolixus* as experimental subjects because, similar to other triatomine species, females exhibit clear and stereotyped discrimination behavior towards undesirable males, which prevent the contact or limit his access to the female's genitalia (Manrique and Lazzari 1994; Roces and Manrique 1996; Pires et al. 2004; Vitta and Lorenzo 2009; Pompilio et al. 2016). The stereotyped female active discrimination behavior exhibited by *R. prolixus* allows measuring female individual preferences against isolated males, a necessary condition for studying female sequential choice.

Under sequential choice scenarios, rejecting a single male can be beneficial if mating opportunities are readily available and the pool of males varies in their quality (Bleu et al. 2012). Thus, the benefits of finding a better quality male may override the cost of rejecting a mating opportunity.

Male availability is a key factor to consider when making a rejection decision under sequential choice scenarios due to the potential lack of future mating opportunities. When potential mates are abundant, females can afford to be choosier as the costs of rejecting males with less preferred traits are small. However, when potential mates are scarce, search costs may preclude high levels of choosiness (Shelly and Bailey 1992; Berglund 1995). Female field crickets *Gryllus lineaticeps* (especially the youngest) spend more time near high male-calling density environments (Atwell and Wagner 2014). Similarly, green tree frog females (*Hyla cinerea*) increase their assessment and acquisition behaviors when they are exposed to playbacks signaling the presence of attractive males (Neelon and Höbel 2017). Female blood-sucking bugs (*R. prolixus*), for example, only reject a courting male when they are exposed to stimuli signaling the presence of more conspecifics (Pompilio et al. 2016), suggesting that the availability of potential mates is strongly considered by females in order to make their mating decisions.

Another crucial factor that may help females to make optimal decisions under sequential choice scenarios is to use current information to estimate the probability of finding a better quality male. To that end, females should be capable of evaluating the quality of potential mates and using it as a reference to accept or reject a single courting male. Previous evidence suggests that female mating decisions may be affected by experience. Mating preferences of female wolf spiders, for example, can be acquired through exposure as subadults to unrelated, sexually active adult males (Hebets 2003). Along the same line, previous acoustic experience appears to affect mating preferences in *Gryllus lineaticeps* females: exposure to

either more attractive songs or more variable songs makes normally unattractive songs even less attractive (Wagner et al. 2001). These results suggest that females use a more complex search rule in which they adjust their searching behavior based on the local distribution of male phenotypes. However, the influence of perceptive and cognitive processes on female mating decision rules is still poorly known (Griggio et al. 2016). Do females adjust the probability of accepting a single courting male based on his absolute quality, or is the quality of the courting male compared with the quality of potential candidates even if they are not currently available?

On this basis, we investigate whether the female decision to accept or reject a single mating opportunity depends exclusively on the quality of the courting male or, is instead affected by the quality of the surrounding males. To that end, naïve females of *R. prolixus* were simultaneously exposed to both a focal male (currently available) and a background male (potentially available). We hypothesize that females are sensitive to the males' nutritional condition since it could be related to their genetic quality. On this basis, prior to the experiment, the nutritional condition of each focal and background male was manipulated to be either low (M<sup>-</sup>) or high (M<sup>+</sup>). Previous work shows that M<sup>+</sup> have more reproductive success than M<sup>-</sup>, evidenced through an increase in the number of fertilized eggs (De Simone 2018). Each female was randomly exposed to one of the following combination of focal and background males (M<sup>-</sup>/M<sup>+</sup>, M<sup>+</sup>/M<sup>-</sup>, M<sup>+</sup>/M<sup>+</sup>, M<sup>-</sup>/M<sup>-</sup>). We analyzed whether and how female rejection behavior is influenced by the absolute and/or relative quality of the courting male. One possibility (fixed template hypothesis) is that females compare each courting male against an innate and fixed template. Thus, the female decision of accepting/rejecting a focal male may depend exclusively on his quality and the quality of a background male should not have any effect. Another possibility (absence of template hypothesis) proposes that females do not have an innate template and their mating decisions depend on external references provided by their current experience. In this case, a female's decision to accept/reject a courting male should depend exclusively on the comparison between the qualities of the focal male and the background male. Specifically, the absence of a template can be inferred from comparing female rejection behavior between focal M<sup>+</sup> and focal M<sup>-</sup> males in situations in which the quality of the background male is equal to the quality of the focal male (M<sup>+</sup> and M<sup>-</sup>, respectively). Under such conditions, the background male does not provide an external different reference that allows females to compare and rank males. Thus, females should respond similarly to focal M<sup>+</sup> and M<sup>-</sup> males. Finally, we consider the possibility that females have an innate template which is not fixed but is adjusted based on experience (flexible template hypothesis). Thus, a female's decision to accept/reject a courting male should be affected by both the

quality of the focal male itself and the quality of the background male.

We hypothesize that rejection behavior evolved as a flexible trait since females may maximize fitness by considering the opportunity/cost of rejecting a mating opportunity.

## Materials and methods

### Insects

*Rhodnius prolixus* used were reared in our laboratory insectary from eggs at  $28 \pm 1$  °C temperature and 30–40% relative humidity, under a 12:12-h (L:D) illumination regime. All instars were fed weekly ad libitum for about 45 min on live hens. During the feeding session, the insects were kept inside a cylindrical acrylic flask (9.5 cm high  $\times$  8.5 cm diameter) with a piece of filter paper as substrate and a nylon mesh as a cover and a floor. Each flask was gently placed in close contact with the hen's skin and therefore insects used the substrate to walk and reach the food source. Insects were sorted by sex after the imaginal ecdysis, keeping virgin adults segregated individually in different plastic containers (3.5 cm high  $\times$  3.0 cm diameter) with a piece of filter paper as substrate until the experiments were performed. Adults were fed for 1 h periodically according to the assays planned (see below). After diuresis (ca. 4 postfeeding hours), insects were weighted and those that fed below 55.6 mg of blood and/or below 81.4% of their weight in blood were discarded (Goodchild 1955; Friend et al. 1965).

Male quality was manipulated through changes in their nutritional condition. High-condition males (M+) were 35-day-old males, fed four times postecdysis on a weekly basis, which were starved for 7 days prior to the experiments. Low-condition males (M-) were 35-day-old males, fed once 5 days postecdysis, which were starved for 28 days prior to the experiments. Females were 12 days old and were starved for 7 days prior to the experiments. Under these conditions, insects were prone to copulate.

Insects were used only once and then discarded. All experiments were carried out during the first hours of their scotophase to match the maximal activity period observed for triatomines (Lazzari 1992). Experiments were performed at a homogeneous illumination intensity of 15 lx, enough to both allow the experimenter to observe the bugs and avoid insect disturbance by high illumination intensity. The temperature of the experimental room was set to  $25 \pm 2$  °C before the beginning of each assay with an electric fan heater, which was turned off before starting each experiment. The relative humidity ranged between 44 and 50%.

### Experimental device

We designed an experimental device to evaluate sequential female mate choice under laboratory conditions. The experimental arena consisted of a circular acrylic box (5 cm height  $\times$  15 cm diameter) that had two acrylic containers (5 cm height  $\times$  3 cm diameter) attached from one of their bases at opposite sides of the box (Fig. 1). The inner base of each container had an odor-permeable removable transparent acrylic partition with holes that allowed the access to visual cues and diffusion of odors, respectively. Thus, three compartments were defined. The box was covered by a removable lid after releasing the insects. A filter paper matching the floor of the arena served as substrate to avoid possible chemical contamination between assays, as it was changed every time new bugs were released.

### Treatment groups

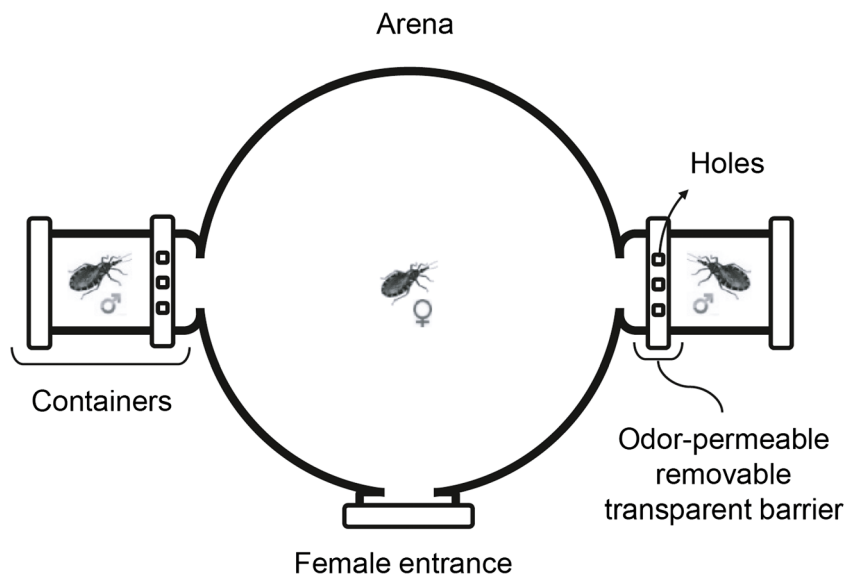
The experiment followed three phases: evaluation, sequential testing, and simultaneous testing. During the evaluation phase, females were simultaneously exposed to two males separated from the female by the transparent barrier of the container; plenty of tiny holes allowed the female to observe and smell the males. During the sequential testing phase, one of the males (hereafter, the focal male) was released, while the other male (hereafter, the background male) remained in the container. Finally, in the simultaneous testing phase, the background male was released as well.

Female acceptance/rejection exhibited to the focal male during the sequential phase (when the background male was still physically inaccessible) was the main measure of female preference. Since the purpose of the experiment was to study whether female preferences are based on the condition of the confronted (focal) male itself or, instead, are affected by the condition of surrounding (background) males, we defined two explanatory variables ("focal male" and "background male") that could take different values (M-/M+). Therefore, the intersexual groups were randomly defined following a factorial design—focal male (M-/M+) and background male (M-/M+)—testing all possible combinations and yielding four experimental groups: M-/M-, M+/M+, M-/M+, and M+/M-.

### Procedure

Before the experiments, insects were weighted to confirm their nutritional condition (Mettler AJ100, Mettler Instrument Corporation, Hightstown, NJ, USA). In each assay, the insects were individually released into the arena, with the two smaller compartments randomly assigned to the focal or background male and the third larger one to the female. The female was released first, the focal male second, and the background male third. To avoid mechanical disturbance that could

**Fig. 1** Diagram of the experimental set up. Insects were tested in a circular arena attached to two containers, separated by an odor-permeable removable transparent barrier. The experimental arena allowed to measure male-female behavioral interactions



trigger a discharge of Brindley's and metasternal glands when released into the arena, each individual was allowed to climb onto a piece of filter paper, which was then placed into each compartment, letting the insects walk freely (Manrique et al. 2006). During the evaluation phase, the female was released and could freely explore the arena for 5 min, including the area close to the inner base of the compartments containing the males. During sequential testing, one of the minor partitions was removed, allowing the female to interact with the focal male. After the first copulatory attempt and the consequent female acceptance/rejection behavior, the background male was released and simultaneous testing began. Thus, copulation occurred exclusively during the simultaneous testing phase (since the sequential testing phase ended immediately after the female response to the first copulatory attempt of the focal male). Note that the female could not mate twice: she either copulated with the focal male or with the background male in the simultaneous testing phase.

Female/male interaction behavior was monitored and recorded (see Pompilio et al. 2016) from the beginning of the evaluation phase until the first copulation occurred in the simultaneous testing phase. We recorded female stereotyped behavior previously associated with rejection of copulation: abdominal movements, flattening, evasion, stridulation, stamping, and/or abdominal raising. The presence/absence of rejection behavior to the first copulatory attempt of the focal male during the sequential testing phase was considered as the main measure of female preference. The female acceptance (i.e., lack of rejection response to the first copulatory attempt) in the sequential testing phase does not necessarily result in copulation, since genitalic contact is not immediate, and once the female accepted or rejected the focal male, the background male was released.

The insect's behavior was recorded using a web camera (Genius FaceCam 1010 HD, KYE Systems Corp., Taipei, Taiwan) placed 35 cm above the arena. After each assay, the insects were carefully removed from the arena with a piece of filter paper to avoid disturbance.

### Data analysis

To assess female preferences, we scored the presence/absence of female rejection behavior during the sequential testing phase regardless of the type of stereotyped behavior exhibited. We compared females' responses of rejection to the first copulatory attempt performed by the focal male in the sequential testing phase across treatments. Note that the focal male was always released in first order and during the sequential phase the background male was present but still physically inaccessible to the female. To study the effects of the nutritional condition of both focal males and background males on the female decision of accepting/rejecting the focal male in the sequential phase, we used a generalized linear model (GLM) with a binary response variable, a logit link, and a binary error distribution, in which the explanatory variables were the nutritional condition of the "focal male (M-/M+)" and the nutritional condition of the "background male (M-/M+)", and the dependent variable was "rejection behavior" (yes/no).

Additionally, we compared across treatments the number of copulations of focal and background males during the simultaneous testing phase using chi-square tests and Bonferroni correction.

We recorded additional information such as latencies to first mounting and copulation between treatments, which were compared using two-way ANOVA. Weight differences between males of different nutritional conditions (M+ and M-)

were confirmed (ANOVA:  $F = 5.25$ ,  $P < 0.0001$ ,  $df = 1$ ,  $N = 80$ ;  $M+ = 0.088 \pm 0.001$  g, mean  $\pm$  SE;  $M- = 0.057 \pm 0.001$  g, mean  $\pm$  SE), with the weight of  $M+$  males being 35.06% higher than that of  $M-$  males. We fitted all models in InfoStat® v2016p (<http://www.infostat.com.ar>).

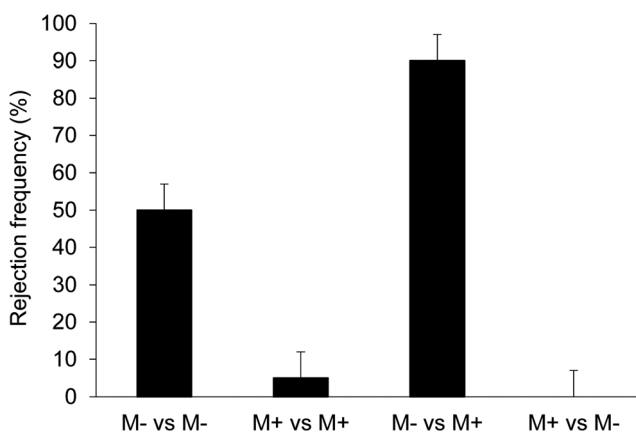
To minimize observer bias, the observer was blind to the treatments when behavioral data were recorded.

**Data availability** The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Results

The main effects evaluated correspond to male-female encounters in the sequential testing phase. As shown in Fig. 2, 50% of rejection behavior was found when both the focal and background males were of low condition ( $M-/M-$ ). The rejection behavior reached a maximum (90%) when the focal male was of low condition and the background male was of high condition ( $M-/M+$ ). Whenever the focal male was of high condition, the female rejection behavior was minimum or null.

The results of the GLM, with focal male ( $M+/M-$ ) and background male ( $M+/M-$ ) as explanatory variables and “rejection behavior” (yes/no) as dependent variable, showed that the condition of both the focal male and the background male significantly affect the rejection behavior by the female ( $P < 0.0001$  and  $P < 0.0023$ , respectively). We found as well a significant interaction between both factors (GLM:  $F = 6.01$ ,  $P = 0.0166$ ,  $df = 1$ ,  $N = 80$ ), meaning that female rejection significantly increases when the focal male is of low condition and when the background male is of high condition. Considering the main effects, the probability of the exhibition of female rejection behavior increased 73% when the focal



**Fig. 2** Percentage (+ SE) of female rejection in the sequential phase according to the nutritional condition ( $M-/M+$ ) of the focal male (release in first order) vs. the background male (release in second order) ( $N = 80$ )

male was  $M-$ , a result that reverted to 3% when the focal male was  $M+$ . On the other hand, when the background male was  $M+$ , the probability to reject the focal male increased 48%, while the probability of rejection decreased to 25% when the background male was  $M-$ .

No statistical differences on latency to first mounting in the sequential testing phase were found between  $M+$  and  $M-$  males (ANOVA:  $F = 0.20$ ,  $P = 0.8933$ ,  $df = 3$ ,  $N = 80$ ). Additionally, we analyzed the frequency of copulation in the simultaneous testing phase (Fig. 3) to study whether copulation was affected by the order in which males were released (focal = first, background = second). To that end, we compared scenarios where both males had the same condition ( $M-/M-$  and  $M+/M+$ ). In both situations, most females copulated with the first male (i.e., the focal male) ( $\chi^2 = 0.92$ ,  $P = 0.3363$ ) (Fig. 3). However, when comparing scenarios in which males' condition differed, two situations occurred. Female always copulated with the first male of high condition when the second male released was of low condition ( $M+/M-$ ). However, when the first male was of low condition, 45% of females copulated with the high-condition male although he was presented second ( $M-/M+$ ) ( $\chi^2 = 11.61$ ,  $P = 0.0007$ ). The number of copulatory attempts did not differ between  $M+$  and  $M-$  in the simultaneous testing phase ( $\chi^2 = 0.10$ ,  $P = 0.7506$ ,  $N = 80$ ).

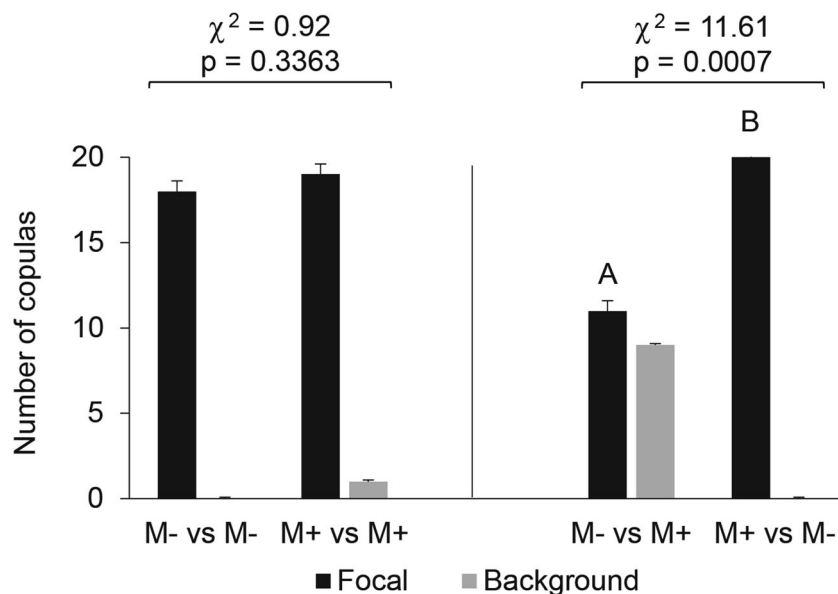
As expected, latency to copulation was marginally longer when the focal male was of low condition and the background male was of high condition, possibly due to the higher frequency of female rejection behavior observed in this treatment (ANOVA:  $F = 2.60$ ,  $P = 0.0581$ ,  $df = 3$ ,  $N = 80$ ).

## Discussion

We found that females of *R. prolixus* are sensitive to differences in the nutritional condition of males, preferring males in better nutritional condition. This conclusion is compatible with the results obtained across the different phases of the experiment. During the evaluation phase, all the females explored each compartment containing both the focal male and the background male, suggesting that they assessed the males individually. When the focal male was released in the sequential testing phase, females showed clear preferences for males of high nutritional condition. However, the probability of accepting or rejecting a focal male depended not only on his nutritional condition but also on the nutritional condition of the background male. When the background male was released in the simultaneous testing phase, most females copulated with the male released first (the focal male) except in the situation in which the background male had a better nutritional condition than the focal male.

Is the nutritional condition a reliable indicator of male quality? Females can use a number of reliable signals in order to

**Fig. 3** Number of copulas (+ SE) of focal and background males in the simultaneous phase for all possible combinations of nutritional condition (M-/M-, M+/M+, M-/M+, and M+/M-). Different letters represent significant differences among groups ( $N = 80$ )



choose the best mate available. Male candidates display their traits through the exhibition of honest cues such as body size, songs, or colors (Zahavi 1975; Andersson 1994). Although previous work has provided insights into the factors affecting female mating preferences, it is less well-known how nutrition-dependent phenotypes influence female mate choice in insects (Xie et al. 2015). In triatomines, this is the first evidence showing female preferences for males in better nutritional condition. Reaching a good nutritional condition in nature is not easy, since resources are scarce and individuals must compete for them. For this reason, we hypothesize that the nutritional condition of a male may be a reliable indicator of genetic quality under certain circumstances (i.e., that the environmental manipulation affect the same traits in the same ways as do alleles that affect nutritional condition, that there is a positive genetic correlation between nutritional condition and fitness). Nutritional condition can also provide females information about direct benefit quality, such as sperm quality or seminal fluid quantity (Fricke et al. 2008; Aluja et al. 2009; Kahrl and Cox 2015; Xie et al. 2015).

Nutritional condition as an indicator of male quality has been frequently explored (e.g., Bonduriansky 2001; Cotton et al. 2004; Bonduriansky et al. 2015). Female Nile tilapia (*Oreochromis niloticus*), for example, prefer the chemical cues of males that are well fed with high-protein diet over those fed with low-protein diet. Females also show a preference for well-fed males, discriminating between the odors with respect to nutritional state, suggesting that they are responding to a food-specific chemical cue (Cardoso Giaquinto et al. 2010). It is therefore likely that nutritional condition is related to the production of pheromones in males.

Studies in insects have manipulated male nutritional condition and examined the effects on males' signals and female choice (Cotton et al. 2004; Abdel-Azim et al. 2018). Andrade

and Mason (2000) found that male scaly cricket (*Ornebius aperta*) on high-nutrient diet were more likely to mate repeatedly and transferred more spermatophores on average than low-diet males (in poor condition). Since courtship and copulatory interactions were always terminated by females, the authors conclude that variation in male repeated mating success may be due to female choice rather than an inability or unwillingness of low-diet males to produce spermatophores. Recently, Xie et al. (2015) found that female ladybirds (*Cryptolaemus montrouzieri*) preferred to mate with males reared under high-nutrition diet regimes, regardless of the nutritional conditions they experienced during their own larval stages, indicating that mate choice of these females is non-random and phenotype dependent. Regarding triatomines, this is the first evidence showing female preferences for males in better nutritional condition.

Extreme differences in nutritional condition may generate low-condition males unable to approach and copulate with females. To avoid this situation, we worked with mild differences in the nutritional condition between high- and low-condition males. Both high- and low-condition males were reared under the same food regime and differed only in the number of days of food deprivation during their adult stage: 7 vs. 28 days, respectively. Note that *R. prolixus* can survive approximately 90 days of starvation and that only after approximately 60 days of food deprivation males are unable to copulate (Khalifa 1950). In fact, we found that all low-condition subjects were capable of copulating and transferring a spermatophore. The results show as well that both high- and low-condition males attempted to copulate with the female in the sequential testing phase and their latencies to mount did not differ, suggesting that the treatment did not affect their courtship behavior. In addition, during the simultaneous testing phase, the number of copulatory attempts did not vary

between the groups. These results mean that both high- and low-condition males were similarly prone to copulate in both sequential and simultaneous testing phases. Moreover, the measure of female preference (acceptance/rejection) was taken once the male mounted the female, meaning that the response of the female could not be affected by the lack of reaction of males.

The fact that females were capable of distinguishing males experiencing different levels of food deprivation shows that nutritional condition affected at least one male trait, since the manipulated variable (days of food deprivation) is not distinguishable per se. As possible mechanisms to explain the results, we speculate that female preferences may be based either on visual cues (such as males' abdomen size), on chemical cues (e.g., differences in pheromone production), or on another unknown variable affected by the nutritional condition of the males. Chemical cues probably constitute an important source involved in mating for triatomines. For *Triatoma infestans* and *Rhodnius prolixus*, pheromones had been associated with male sexual aggregation. It was shown that females are indifferent to the same released compounds (Manrique and Lazzari 1995; Crespo and Manrique 2007; Pontes and Lorenzo 2012). The reason may lay in the fact that females simply do not aggregate, but are capable of detecting and considering olfactory and or visual clues in order to make a choice decision.

Do females adjust their mating decisions based on the quality of potential mates? Female choice was historically assumed to be based on absolute preferences, where females assess males independently, assigning a fixed value to each male that they encounter (Real 1990; Crowley et al. 1991; Jennions and Petrie 1997). Further evidence suggests instead that under simultaneous choice scenarios females may perform a comparative evaluation of prospective mates. Male candidates, in these cases, were presented during the evaluation phase either simultaneously or sequentially, but tested in all cases under simultaneous choice scenarios (Bakker and Milinski 1991; Reaney 2009; Locatello et al. 2015). Moreover, the studies investigating female sequential choice also suggest that females adjust their responses based on their past experience (Bateman et al. 2001; Pitcher et al. 2003). In these cases, however, the second encounter occurred once the female copulated with the first male, meaning that the motivation for copulation dropped systematically during the testing phase (and during the first encounter the female could not anticipate the quality of the potential following candidate(s)). Our experimental device allowed testing the precopulatory reaction of females to a single available male, while she could assess the presence and quality of another potential candidate. The results obtained in the sequential testing phase support the idea that the decision of accepting or rejecting a male depends on both the quality of the courting male and the quality of the potential mates. This result is compatible with the idea that

females compare each courting male against a template and that such a template is adjusted based on the quality of the current males potentially available. The females used in the experiment were naïve and did not have previous experience with males. Nevertheless, they were capable of differentiating between high- and low-quality males since they behaved differently when exposed to M-/M- and M+/M+ scenarios, suggesting that the template is innate. On the other hand, we showed that such a template is flexible since female rejection behavior is adjusted according to the quality of potential mates. Previous indirect results are consistent with the idea that, with higher availabilities of mates, females are often more discriminating (Berglund et al. 2005; Willis et al. 2011; Atwell and Wagner 2014). Besides, it was found that females invest more in acquisition and searching behavior when exposed to stimuli which signal the presence of high-quality males (Neelon and Höbel 2017). We found that the quality of potential mates affects female probability of accepting or rejecting a courting male. Thus, females adjust their choice based on the distribution of the quality of males present. This result has direct implications for the evolution of male sexual traits since conditions that cause females to mate less or more discriminately may affect the strength of sexual selection and, as a consequence, the rate of spread of preferred male traits (Poulin and Vickery 1996; Kudo and Karino 2012).

On this basis, we speculate that rejection behavior evolved as a flexible trait that allows females to maximize fitness by considering the opportunity/cost of rejecting a mating opportunity. This behavioral flexibility may help females to optimize and maximize the benefits of their mating decisions.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** We did not observe any harmful effects associated to our experimental manipulation. At the end of the experiment, the insects were returned to their breeding cages and the hens to the chicken coop. All animals were handled according to the biosafety rules from the Servicio de Higiene y Seguridad of the Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina.

**Informed consent** Not applicable.

## References

- Abdel-Azim MM, Aldosari SA, Shukla P (2018) Factors influencing mating behavior and success in the red palm weevil, *Rhynchophorus ferrugineus* Olivier (Coleoptera: Dryophthoridae). *Neotrop Entomol*. <https://doi.org/10.1007/s13744-018-0592-9>
- Aluja M, Rull J, Sivinski J, Trujillo G, Pérez-Staples D (2009) Male and female condition influence mating performance and sexual receptivity in two tropical fruit flies (Diptera: Tephritidae) with contrasting life histories. *J Insect Physiol* 55:1091–1098
- Andersson MB (1994) Sexual selection. Princeton University Press, Princeton, New Jersey
- Andrade MCB, Mason AC (2000) Male condition, female choice, and extreme variation in repeated mating in a scaly cricket, *Ornebius aperta* (Orthoptera: Gryllidae: Mogoplistinae). *J Insect Behav* 13:483–497
- Atwell A, Wagner WE (2014) Female mate choice plasticity is affected by the interaction between male density and female age in a field cricket. *Anim Behav* 98:177–183
- Bakker TCM, Milinski M (1991) Sequential female choice and the previous male effect in sticklebacks. *Behav Ecol Sociobiol* 29:205–210
- Bateman PW, Gilson LN, Ferguson JWH (2001) Male size and sequential mate preference in the cricket *Gryllus bimaculatus*. *Anim Behav* 61:631–637
- Berglund A (1995) Many mates make male pipefish choosy. *Behaviour* 131:213–218
- Berglund A, Widemo MS, Rosenqvist G (2005) Sex-role reversal revisited: choosy females and ornamented, competitive males in a pipefish. *Behav Ecol* 16:649–655
- Bleu J, Bessa-Gomes C, Laloia D (2012) Evolution of female choosiness and mating frequency: effects of mating cost, density and sex ratio. *Anim Behav* 83:131–136
- Bonduriansky R (2001) The evolution of male mate choice in insects: a synthesis of ideas and evidence. *Biol Rev* 76:305–339
- Bonduriansky R, Mallet MA, Arbuthnott D, Pawlowsky-Glahn V, Egozcue JJ, Rundle HD (2015) Differential effects of genetic vs. environmental quality in *Drosophila melanogaster* suggest multiple forms of condition dependence. *Ecol Lett*. <https://doi.org/10.1111/ele.12412>
- Cardoso Giaquinto P, da Silva Berbert CM, Delicio HC (2010) Female preferences based on male nutritional chemical traits. *Behav Ecol Sociobiol* 64:1029–1035
- Cotton S, Fowler K, Pomiankowski A (2004) Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proc R Soc Lond B* 271:771–783
- Crespo JG, Manrique G (2007) Mating behavior of the haematophagous bug *Triatoma infestans*: role of Brindley's and metasternal glands. *J Insect Physiol* 53:708–714
- Crowley PH, Travers SE, Linton MC, Cohn SL, Sih A, Craig Sargent R (1991) Mate density, predation risk, and the seasonal sequence of mate choices: a dynamic game. *Am Nat* 137:567–596
- De Simone GA (2018) Factores y reglas de decisión involucrados en la elección de pareja sexual en el insecto hematofago *Rhodnius prolixus* (Hemiptera: Reduviidae). Dissertation (Ph D. Thesis), Universidad de Buenos Aires
- Fricke C, Bretman A, Chapman T (2008) Adult male nutrition and reproductive success in *Drosophila melanogaster*. *Evolution* 62:3170–3177
- Friend W, Choy C, Cartwright E (1965) The effect of nutrient intake on the development and the egg production of *Rhodnius prolixus* Stål (Hemiptera: Reduviidae). *Can J Zool* 43:891–904
- Gabor CR, Halliday TR (1997) Sequential mate choice by multiply mating smooth newts: females become more choosy. *Behav Ecol* 8:162–166
- Goodchild AJP (1955) Some observations on growth and egg production of the blood-sucking Reduviids, *Rhodnius prolixus* and *Triatoma infestans*. *Physiol Entomol* 30:137–144
- Griggio M, Hoi H, Lukasch B, Pilastro A (2016) Context-dependent female preference for multiple ornaments in the bearded reedling. *Ecol Evol* 6:493–501
- Hebets EA (2003) Subadult experience influences adult mate choice in an arthropod: exposed female wolf spiders prefer males of a familiar phenotype. *PNAS* 100:13393
- Janetos AC (1980) Strategies of female mate choice: a theoretical analysis. *Behav Ecol Sociobiol* 7:107–112
- Jennions MD, Petrie M (1997) Variation in mate choice and mating preferences: a review of causes and consequences. *Biol Rev* 72:283–327
- Kahril AF, Cox RM (2015) Diet affects ejaculate traits in a lizard with condition-dependent fertilization success. *Behav Ecol* 26:1502–1511
- Khalifa A (1950) Spermatophore production and egg-laying behaviour in *Rhodnius prolixus* Stål (Hemiptera; Reduviidae). *Parasitology* 40:283–289
- Kudo H, Karino K (2012) Short-term change in male sexually selected traits and female mate preference in the guppy *Poecilia reticulata*. *Ichthyol Res* 59:1–7
- Lazzari CR (1992) Circadian organization of locomotion activity in the haematophagous bug *Triatoma infestans*. *J Insect Physiol* 38:895–903
- Locatello L, Poli F, Rasotto MB (2015) Context-dependent evaluation of prospective mates in a fish. *Behav Ecol Sociobiol* 69:1119–1126
- Manrique G, Lazzari CR (1994) Sexual behaviour and stridulation during mating in *Triatoma infestans* (Hemiptera: Reduviidae). *Mem Inst Oswaldo Cruz* 89:629–633
- Manrique G, Lazzari CR (1995) Existence of a sex pheromone in *Triatoma infestans* (Hemiptera: Reduviidae): I. Behavioural evidences. *Mem Inst Oswaldo Cruz* 90:645–648
- Manrique G, Vitta ACR, Ferreira RA, Zani CL, Unelius CR, Lazzari CR, Diotaiuti L, Lorenzo MG (2006) Chemical communication in Chagas disease vectors. Source, identity and potential function of volatiles released by the metasternal and Brindley's glands of *Triatoma infestans* adults. *J Chem Ecol* 32:2035–2052
- Murphy CG, Gerhardt HC (2002) Mate sampling by female barking treefrogs (*Hyla gratiosa*). *Behav Ecol* 13:472–480
- Neelon DP, Höbel G (2017) Social plasticity in choosiness in green tree frogs, *Hyla cinerea*. *Behav Ecol* 28:1540–1546
- Pires HHR, Lorenzo MG, Lazzari CR, Diotaiuti L, Manrique G (2004) The sexual behaviour of *Panstrongylus megistus* (Hemiptera: Reduviidae): an experimental study. *Mem Inst Oswaldo Cruz* 99:295–300
- Pitcher TE, Neff BD, Rodd FH, Locke R (2003) Multiple mating and sequential mate choice in guppies: females trade up. *Proc R Soc B Biol Sci* 270:1623–1629. <https://doi.org/10.1098/rspb.2002.2280>
- Pompilio L, González Franco M, Chisari LB, Manrique G (2016) Female choosiness and mating opportunities in the blood-sucking bug *Rhodnius prolixus*. *Behaviour* 153:1863–1878
- Pontes GB, Lorenzo MG (2012) Female metasternal gland odours mediate male aggregation in *Rhodnius prolixus*, a triatomid bug. *Med Vet Entomol* 26:33–36
- Poulin R, Vickery WL (1996) Parasite-mediated sexual selection: just how choosy are parasitized females? *Behav Ecol Sociobiol* 38:43–49
- Real LA (1990) Search theory and mate choice. I. Models of single-sex discrimination. *Am Nat* 136:376–405
- Real LA (1991) Animal choice behavior and the evolution of cognitive architecture. *Science* 253:980–986
- Reaney T (2009) Female preference for male phenotypic traits in a fiddler crab: do females use absolute or comparative evaluation? *Anim Behav* 7:139–143



- Roces F, Manrique G (1996) Different stridulatory vibrations during sexual behaviour and disturbance in the blood-sucking bug *Triatoma infestans* (Hemiptera: Reduviidae). *J Insect Physiol* 42:231–238
- Schäfer MA, Uhl G (2005) Sequential mate encounters: female but not male body size influences female remating behavior. *Behav Ecol* 16:461–466
- Shelly TE, Bailey WJ (1992) Experimental manipulation of mate choice by male katydids: the effect of female encounter rate. *Behav Ecol Sociobiol* 30:277–282
- Vitta ACR, Lorenzo MG (2009) Copulation and mate guarding behavior in *Triatoma brasiliensis* (Hemiptera: Reduviidae). *J Med Entomol* 46:789–795
- Wagner WE Jr, Smeds MR, Wiegmann DD (2001) Experience affects female responses to male song in the variable field cricket *Gryllus lineaticeps* (Orthoptera, Gryllidae). *Ethol* 107:769–776
- Wiegmann DD, Angeloni LM, Seubert SM, Wade JG (2013) Mate choice decisions by searchers. *Curr Zool* 59:184–199
- Willis PM, Ryan MJ, Rosenthal GG (2011) Encounter rates with conspecific males influence female mate choice in a naturally hybridizing fish. *Behav Ecol* 22:1234–1240
- Wittenberger JF (1983) Tactics of mate choice. In: Bateson P (ed) *Mate choice*. Cambridge University Press, New York, pp 435–447
- Xie J, De Clercq P, Zhang Y, Wu H, Pan C, Pangb H (2015) Nutrition-dependent phenotypes affect sexual selection in a ladybird. *Sci Rep* 7:159–170
- Zahavi A (1975) Mate selection—a selection for a handicap. *J Theor Biol* 53:205–214