

## Intense sexual conflict affects the female choice strategy in the gregarious desert locust

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### Abstract

The antagonistic sexual coevolution of the sexes (i.e., sexual conflict) is a key theme in the study of the evolution of mating systems. Specifically, studying the modifications of sexual behavior in the context of ecological conditions is fundamental for understanding the formation of a sexual system. The extremely crowded situation that prevails in the mating system of gregarious desert locusts is expected to result in a fierce sexual conflict. The goal of this work was to investigate core aspects of the sexual conflict during pre-copulatory interaction: (i) Female choosiness, (ii) preference functions (mate sampling strategy), (iv) antagonistic armament, (v) male endurance as an indicator of his fitness. We tested the pre-copulatory behavior of the gregarious desert locust (*Schistocerca gregaria*) under two distinguished conditions: (1) No-choice - a pair interaction (with no intra-male rivalry), and (2) choice – a female with two rival males. We investigate the sexual conflict in *S. gregaria* by ethological-based quantification, focusing on related behaviors. Our findings provide evidence for an intense conflict between the sexes in *S. gregaria*. Further, the results show that the pre-copulatory interaction is highly oriented by female choice. Also, our findings suggest that gregarious females estimate their potential mates using the 'adjustable internal acceptance threshold' sampling strategy. Accordingly, our results suggest that the ability of males to overcome female resistance serves as an indication of the male's quality (i.e., a trait under choice). Last, this paper provides both qualitative and numerical model of a sexual conflict during pre-copulatory interaction in locusts. This study elucidates the behavioral components of the sexual conflict in locusts and contributes to the current understanding of the eco-evolutionary contexts of sexual conflicts.

**Keywords:** Sexual conflict, Mating system, Mate preference, Sampling strategy, Locusts, *Schistocerca gregaria*.

## Introduction

A central concept in the theory of sexual selection is the strong asymmetry in the genetic interest of the partners, known as 'sexual conflict' (Parker 1979; Rice 1996). The conflict of interest exists when one or both partners mate multiply and may advance mating strategies that promote the fitness of one partner but deteriorate that of the other (Janetos 1980; Wittenberger 1983; Real 1990). Co-evolutionarily, though, behavior that reflects a better adaptation of the one sex is followed by a counter-adaptive behavioral response of the other (Arnqvist and Rowe 2002a; Hollis et al. 2019; Rice 1992).

Historically, since Darwin (1897) and Wallace (1892), the study of sexual selection constitutes two main aspects (i) mate choice and (ii) intra-sexual competition for a mate. The mechanisms underlying mate choice selectively favor a mate with a specific sexual attribute that reflects the sire's higher quality or higher reproductive potential (Arnqvist & Rowe 1995; Gonzales-Eriksson et al. 2021; Sullivan 1994). Active choice of mate includes an array of decision-making (Trivers 1972), taking into account the trade-off between gaining a high-quality partner vs. the costs associated with choosing (e.g., energy, time, predation risk, and more) (Gibson & Bachman 1992). The preference of a certain sire over others provides the foundation for sexual conflicts, whereby the interest of the unchosen individuals differs from that of the choosers.

The mechanism of mate preference includes two major aspects (Jennions & Petrie 1997): (1) Choosiness – the efforts dedicated by an individual to mate assessment in a population, and (2) preference functions - a preferability procedure by which an individual determines the quality of a potential mate. The several strategies that have been proposed to describe the process of mate choice (Janetos 1980; Parker 1983; Real 1990; Wittenberger 1983) may be classified into four main categories: (i) *Passive choice* – choosing a mate having the most intense advertising trait, ornament or armament, (ii) *random strategy* – mating with the first, randomly encountered mate, (iii) *threshold strategy* – mating with the first mate having a trait that exceeds the chooser internal threshold; (iv) *comparison strategy* – comparing the trait of choice among multiple candidates, and accepting the mate having the most preferred trait.

The desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae), may serve as a model animal for the effect of environmental conditions on the severity of the sexual conflict. The life history of locusts is characterized by density-dependent phenotypic plasticity, known as polyphenism (Uvarov 1966; Pener & Simpson 2009). Locusts' phenotypic spectrum is confined between two extreme phases, gregarious and solitary. When in the solitary phase, the locust mating system is characterized by very low population density (<5 individuals/ha, Uvarov 1977), resulting in a low likelihood of intra-male competition or multiple matings (Ely et al. 2006). In contrast, when in the gregarious phase, the population is highly crowded with a promiscuous

mating system (Hunter-Jones 1960) and a high intra-male rivalry for access to females during pre-copulation (i.e., pre-mounting and mounting stages) as well as during post-copulation (i.e., mate-guarding) (Otte 1970; Seidelmann & Ferenz 2002). The pre-copulatory behavior of gregarious males is characterized by stealth approaching the female, followed by repeated mounting attempts and possibly copulation (Popov 1958; Loher 1959). The female's rejection is shown by walking/jumping away from the approaching male, and if failed, she attempts to dislodge the mounted male from her back (Odhiambo 1966; Strong & Amerasinghe 1977). At the same time, the mounting male may overcome the female's rejection by using defensive behavior (e.g., avoiding or blocking elements, Golov et al. 2018a).

The sexual interactions of gregarious *S. gregaria* combine both female remating (polyandrous mating system) and strong intra-male competition, thus, an intense sexual conflict is expected (Hammerstein and Parker 1987; Andersson & Simmons 2006). Here, we studied the *S. gregaria* sexual conflict and choice of mate, focusing on the pre-copulatory behaviors, based on the comprehensive description by Golov et al. (2018a).

The study is aimed at (1) examining the antagonistic behaviors of the sexes at the pre-copulatory stage, (2) elucidating the mate preference tactics used by gregarious females, emphasizing *choosiness* and *preference functions*, (3) identifying a male trait that assists mate-choice of females during pre-copulation.

## Material & Methods

### *Animals*

An ongoing colony of the desert locusts, *Schistocerca gregaria* (Forskål 1775), has been kept in Tel Aviv University, Israel, under crowded conditions of 100-160 individuals in 60L aluminum cages. Neonates, offspring of gregarious females, were collected within 2 h of hatching and reared in gregarious conditions until adulthood (Geva et al. 2010). Cages with locusts were maintained under a constant temperature (c. 37°C.) and a light cycle of 12:12 D:L. Locusts were provided daily with fresh wheat leaves and dry oats. Plastic caps (300cc) filled with moist sand were used for oviposition substrates.

Locusts were sexed 48-h after the last molting (based on their color and genitalia), and males and females were kept in different cages. Only virgin adult locusts were used in the experiments (for details, see Golov et al. 2018a). Sexual maturation of gregarious locusts was defined as 12-14-days-old for males and 18-20 day-old for females (Norris 1954).

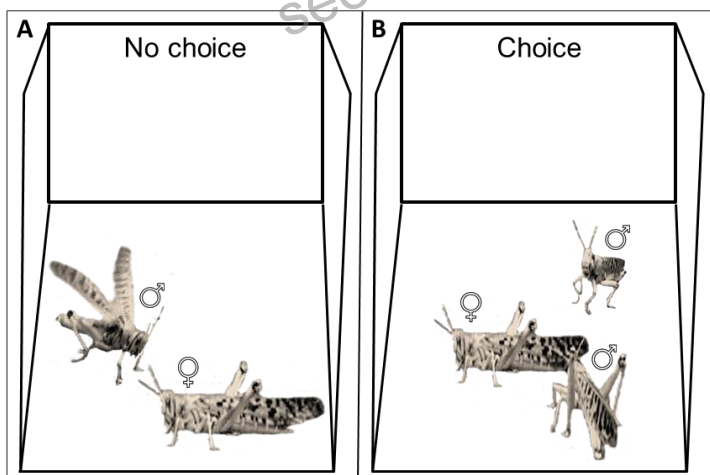
### *Experimental design*

The experimental procedure was carried out as described in Golov et al. (2018a). Briefly, sexually matured locusts were randomly taken from the rearing cages and placed individually in a 50mL test tube. Locusts were then introduced into an experimental cage, previously divided into

two equal-size cells by an opaque plastic partition (cell's dimensions: 14x13x24 cm). Each cell contained one male or one female. After a short acclimation time (ca. 5 min), the temporary opaque plastic between the cages was carefully removed, and the male and female could see each other. At this point, the sexual interactions of the locusts were recorded using a SONY HDR-PJ820E video camera for a maximum of 3 hours, or until copulation had occurred.

### *Female mate choice and intra-male competition*

Male biased operational sex ratio (i.e., the ratio of sexually active males and females) may affect the sexual behavior of both males and females; females have the option to choose among many males, while males compete with other males for the access to a receptive female. To study the role of female choice on the sexual conflict in gregarious locusts, we conducted two treatments: (1) No-choice treatment (N=45) – with a single male and a single female in each of the cells above. (2) A choice treatment (N=37) – placing a pair of rival males in one cell and a single female in the other (**Fig. 1**). The effect of the male body size on his mating probability was tested by placing two rival males that differed in their weights ( $\Delta_{weights} > 0.5 g$ ) (Digital Balance, Precisa 125 A SCS). The first male to mate with the female was defined as a "winner" and his rival as a "loser."



**Fig. 1.** Experimental setup. **A.** No choice treatment – a pair of a male and a female. **B.** Choice treatment – one female and two males.

### *Data processing*

Videos recording the locusts' sexual interactions were processed using BORIS software (Friard and Marco 2016). For each of the treatments, no-choice and choice, we measured (a) *rate of mating* – the number of interactions that ended in mating, divided by the total number of interactions, and (b) *interaction time* – the time (min) from the beginning of the observation until mating.

The behavioral tracking was based on the methodology described in Golov et al. (2018a), focusing only on the behavioral elements strongly associated with sexual conflict (see **Table 1**).

Behavioral quantifications during the pre-mounting stage were conducted only during 'sexual encounter', i.e. when a male and a female are located no more than 10 cm from each other. Quantification of sexual behaviors was based on three main parameters: (1) *Frequency*, (2) *occurrence probability*, and (3) *responding time* (**Table 2**, 'Descriptive parameters'). These parameters used to quantified all the behavioral parameters of this study (**Table 2**, 'Behavioral parameters').

**Table 1.** The repertoire of behavioral elements associated with sexual selection in the desert locusts.

		Conflict behavioral elements	
Stage	Sex	♂	♀
Premounting		<b>Mounting attempt via:</b> <ul style="list-style-type: none"> <li>▪ Climbing</li> <li>▪ Jumping</li> </ul>	<b>Rejection response:</b> <ul style="list-style-type: none"> <li>▪ Walking away</li> <li>▪ Jumping away</li> </ul>
	Mounting	<b>Prevailing rejection via:</b> <ul style="list-style-type: none"> <li>▪ Avoidance</li> <li>▪ Blocking</li> </ul>	<b>Direct rejection via:</b> <ul style="list-style-type: none"> <li>▪ Jumping</li> <li>▪ Kicking</li> </ul>
<ul style="list-style-type: none"> <li>▪ Lifting attempt</li> </ul>		<b>Indirect rejection via:</b> <ul style="list-style-type: none"> <li>▪ Abdominal bending</li> <li>▪ Abdominal grounding</li> </ul>	

**Table 2.** Parameters used to quantify the sexual behavior of the desert locusts.

Descriptive parameters			
Parameter		Description	
▪	Frequency	The counts (cumulative) of a displayed element.	
▪	Occurrence probability	The Boolean scoring of the displayed element (1 if displayed, 0 if not).	
▪	Responding time	The time (sec) took the female to respond to the male's successful mounting, using a rejection element.	
Behavioral parameters			
Stage	Sex	Parameter	Description
Pre-mounting	♂	Mounting effort	Frequency of mounting attempts made by a male.
	♂	Failure rate	Proportional mean of unsuccessful mounting attempts among all mounting attempts.
	♀	Rejection rate	Proportional mean of pre-mounting rejections among all male's mounting attempts.
	♀	Choosiness level	Three classification levels based on a Boolean scoring given according to the display of female's rejection in each of the two precopulatory stages.
Mounting	♂	Mating effort	Frequency of all male's mounts.
	♀	Direct rejection rate	Frequency of the female's direct rejection among all mounts.
	♀	Rejection phenotypes	Classification of choosy females (all showed a direct rejection) into two groups of rejection: (i) compromising and (ii) Stubborn.
	♀	Responding time	Elapsing time (sec) from the initiation of mounting until the display of a rejection behavior.
	♂	Endurance	Frequency of direct rejections experienced by a male (jumping and kicking) divided by its mounts' frequency.

Detailed description of all parameters is given in the chapter of in 'Material & methods'

### The Conflict

#### Female rejection vs. male mating success

The inherent conflict between the male mating success and the female choice was explored in the pre-copulatory stages: pre-mounting and mounting. In the pre-mounting stage, we tested the sexual interactions during the male's attempt to mount successfully on the female's back. The total *accumulated mounting attempts* made by the male used to define his '*mounting effort*'. In each of his mounting attempts (by climbing or jumping), we measure the *occurrence probability* of '*male failure*' (unsuccessful mounting attempts) and '*female rejection*' (based on her rejection display of walking and jumping away). We defined the '*male failure rate*' and '*female rejection rate*' as the *proportional mean* of their *occurrence probabilities*. In the mounting stage, in each mount, we define the '*male mating effort*' as the *frequency* of all mounts of a male and the '*female direct rejection rate*' as the *frequency* of the two direct rejecting elements (jumping and kicking) among all mounts.

#### *Female's choosiness level*

The level of female choosiness was categorized based on four behavioral elements: (1) walking or (2) jumping away from the male in the pre-mounting stage, and (3) jumping or (4) kicking the male during the mounting stage. The choosiness level was assigned by the robust approach of the *occurrence probability* (Boolean scoring) as follows: (i) Low (0) - females who showed no rejection behavior in both pre-mounting and mounting stages. (ii) Moderate (1) - females who showed rejection behavior only during one of the stages. (iii) High (2) – females that displayed rejection behavior during both stages. The choosiness level was determined separately for each female in the two treatments, no-choice (N=45 females) and choice (N=47 females).

#### *Mounting stage as a major sexual conflict point*

Specific attention was given to the sexual interactions during the mounting stage, including the female's rejection level, the antagonistic behavior, and the male endurance.

#### *Rejection phenotypes*

Among all mating females that directly rejected the mounting males (no choice: N=24 out of 31; choice: N=22 out of 25), we measured the *frequency* of mounts for each female. The females were classified into two phenotypes: (i) *Stubborn* - females displaying direct rejection in all mounts, including the last, and (ii) *compromising* - receptive females that did not reject the last males.

#### *Antagonistic behavior*

Behavioral elements displayed during the mounting stage (female: 4 elements; male: 3 elements, see Table 1) were sorted into two categories based on the outcome of the interaction:

(i) The *outcome of the mount* - dislodgement, or mating, and (ii) the experimental treatments - "no choice" and "choice." For each element, the '*rate of occurrence*' was measured as the sum of *occurrence probabilities* (Boolean value per mount), divided by the frequency of all male's mounts frequency (i.e. '*mating effort*').

### *Male endurance*

To assess the males' ability to overcome female rejections, we define '*male endurance*' by calculating the '*rate of direct rejection*' for each male as the weighted average of the total frequencies of direct rejections (jumping and kicking) per mount, experienced by males during all of their mounts. As males' ability to challenge female rejections may be due to a prolonged response of the female, we measured the time elapsed (sec) from the initiation of mounting until the display of rejection behavior.

### *Sexual conflict at the population level*

The degree of the sexual conflict during the various sexual interactions was estimated at the population level using a multivariate approach. For each sexual interaction, the degree of sexual conflict was calculated by combining different parameters in the two pre-copulatory stages: (i) male '*mounting effort*', '*male failure rate*' and '*female rejection rate*'. (ii) '*male mating effort*', '*male endurance*', and the *outcome of the sexual interaction* (i.e., whether copulation has occurred or not).

### *Statistical analysis*

The mating rate in the no-choice treatment (N=45) was compared to that in the choice treatment (N=37) using the two-sided Fisher exact test. The time (min) of interactions that ended in copulation in the no-choice treatment was compared to that in the choice using the two-sided Mann-Whitney U test ( $\alpha=5\%$ ; no-choice: 31 out of 45, choice: 27 out of 37). The analysis of the inherent conflict was done using the *Pearson Correlation test* ( $\alpha=0.05$ ): (1) during the pre-mounting stage – the association between the '*male failure rate*' and the '*female rejection rate*' (no-choice: N=45, Choice: N=68 out of 74, 6 losers displayed no mounting attempt). (2) during the mounting stage: '*female direct rejection rate*' and the '*male mating effort*' (no-choice: N=44 out of 45, 1 excluded outlier, choice: N=58 out of 74, 16 males did not mount the females).

### *Female choosiness level*

Females' choosiness level (no-choice: N=45, Choice: N=37) was analyzed on two scales: (i) at a small scale – to test whether the within-distribution is different from uniform. The analysis was done for each classified *choosiness level* (high, moderate, and low) and treatment (no choice and choice)(G-test of goodness of fit,  $DF=2$ ,  $\alpha=0.05$ ). (ii) At a large scale – we compared the distribution of the choosiness levels between the no choice and the choice treatments, using the two-sided

Cochran–Armitage Chi-Square test for trend. The Cochran–Armitage test is a variation of the chi-square test, designed to compare the proportional distribution of two-levels data (Cochran 1954; Lachin 2011).

### *Mounting stage as a major mate selection point*

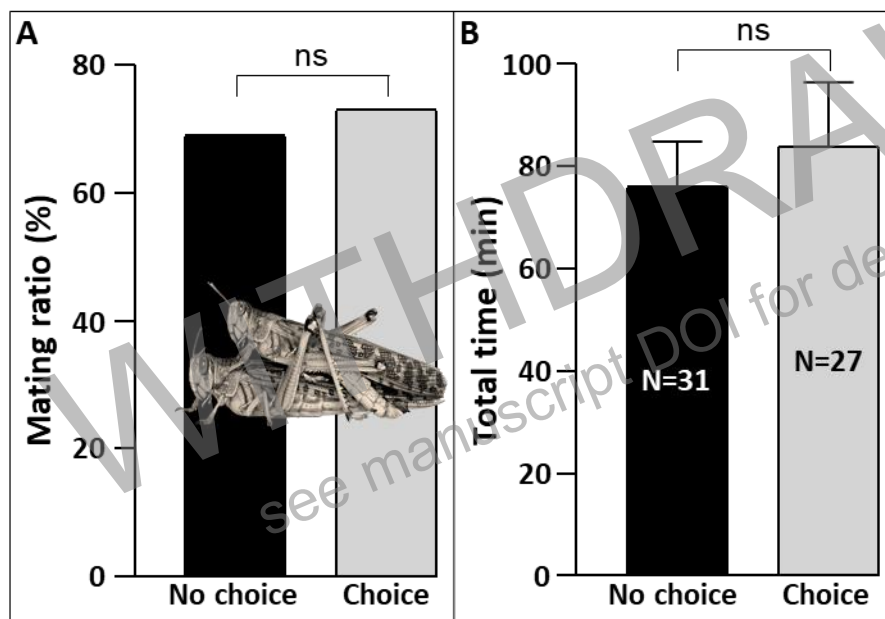
The conflict during mounting includes three different aspects: (1) Rejection threshold – To test the female's rejection threshold, we analyzed the proportional distribution of the two phenotypes (*stubborn* and *compromising*) on a small scale - G-test of goodness of fit, and a large scale - two-sided Cochran–Armitage Chi-Square. The rejection threshold was tested by comparing the proportional distribution of the two rejection phenotypes between the two treatments, no choice and choice, using the Cochran–Armitage Chi-Square test for a trend. This analysis included only mated females that responded with direct rejection towards the mounting male (No choice: N=22, *stubborn*, N=12, *compromising* N=10; choice: N=24, *stubborn*, N=14, *compromising*, N=8). (2) The analyses of the antagonistic behavior included three-independent variables, (i) the treatment (no choice and choice, (ii), the outcome of the male's mounting (dislodgement and mating), and (iii) the behavioral element. Interactions involving highly receptive females who showed none of the four rejection elements (jumping, kicking, abdominal grounding and bending) were excluded from this analysis (No choice: N= 39 out of 45; Choice: N=33 out of 37). Five competing males had mated with receptive females at the first attempt and 16 out of the 27 losers did not mount the female. These males were excluded from the analysis. In order to prevent a violation of statistical assumptions, only sexual interactions that involved both a mating event and the male's dislodgement were used in the statistical analyses (i.e., a pair analysis). For each sex, the analysis was done using a three-factorial non-parametric method of aligned ranks transformation (ART) *repeated ANOVA*, followed by the post hoc Dunn's test for multiple comparisons (Females: no choice, N=16 out of 39; choice, N=14 out of 33; Males: No choice, N=16 out of 39; Choice, N=10 out of 22 winners). (3) Male endurance against the direct female rejection included the analysis of two parameters: the male endurance and the female responding time (sec). The mounts were sorted by the outcome (dislodgement or mating) in each treatment (no choice dislodgement: N=35, mating: N=14, and choice dislodgement: N=47, mating: N=14). The two parameters were analyzed using the two-factorial non-parametric repeated method ART ANOVA and *post hoc Dunn's test* (females=males: no choice, N=10 out of 31 mated interactions; choice, N=11 out of 27 mated interactions). Lastly, investigating the intensity of the sexual conflict at the population level was done by using clustering analysis of K-Means clustering algorithm (no-choice: N=45, choice: N= 68 out of 74, 6 losers that displayed no mounting attempt excluded from this analysis) using 9 parameters. An optimal number of clusters was achieved using the Monte Carlo method's cubic



clustering criterion (CCC) technique. All analyses were conducted using JMP® Pro14, SAS Institute, and GraphPad Prism version 8.0 for Windows.

## Results

The pre-copulatory behavior of gregarious locusts was tested in two treatments: a no-choice (one male and one female, N=45) and a choice (two males and one female, N=37). No difference was found in the Rate of mating (Fig. 2A, two-sided Fisher exact test,  $p(v)>0.05$ ) and the time until mating (Fig. 2B, Mann-Whitney U test,  $p(v)>0.05$ ) between the no-choice and the choice treatments.



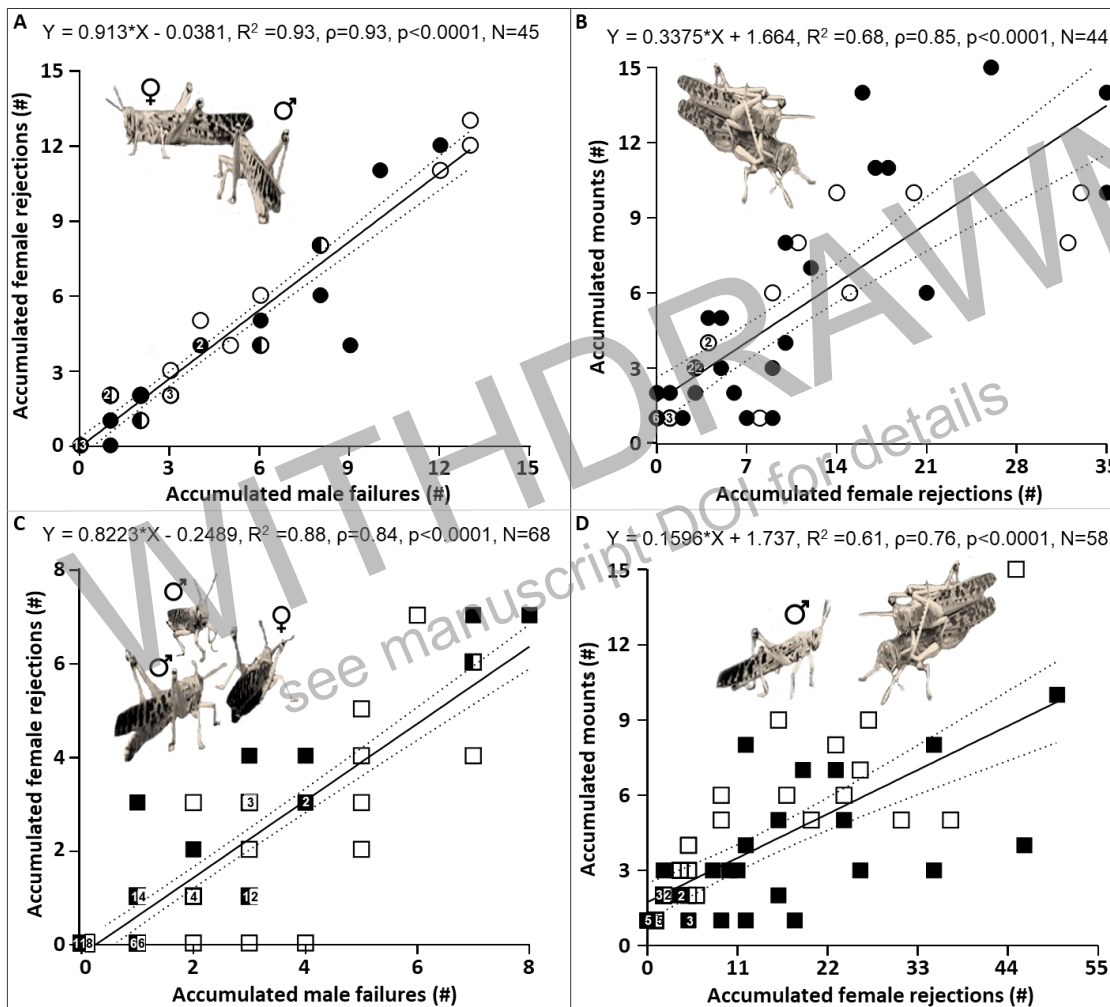
**Fig. 2.** Pre-copulatory interactions of gregarious locusts in no-choice and choice conditions. Black columns – No-choice (one male and one female), gray columns – Choice (two males and one female). **A.** Mating ratio (number of sexual interactions that ended in copulation out of the total number of interactions). No-choice (N=45), choice (N=37). Two-sided Fisher exact test ( $\alpha=5\%$ ). **B.** Elapsing time (min) of interactions that ended in copulation (No-choice: 31 out of 45, Choice: 27 out of 37). Two-sided Mann-Whitney U test ( $\alpha=5\%$ ). In both **A** & **B** the difference between groups is insignificant,  $p(v)>0.05$ .

## The Conflict

### Female rejection vs. male mating success

Measuring the intensiveness of the sexual conflict in a situation of female choice vs. no-choice a significant linear relationship between males' mating success and female rejection was found in the two pre-copulatory stages (Spearman correlation  $\rho$ -test,  $p(v)<0.0001$ ) (Fig. 3). 'Female rejection rate' varied greatly, yet the vast majority of the females respond to males' mating attempt with an overt rejection. Notably, during the pre-mounting stage (Fig. 3-A and C), the

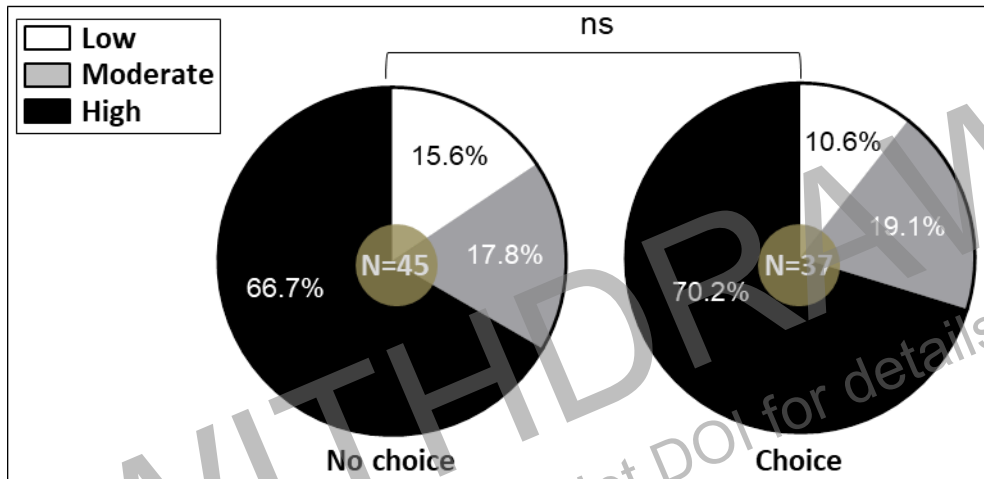
strength of the relationship between the two Boolean parameters, '*male failure rate*' and '*female rejection rate*' is not perfectly fit ( $R^2 < 1.0$ ) due to the failure of some males to successfully mount the receptive females (values below the regression lines). The proportion of these events was higher in the *choice* treatment than in the *no choice* treatment, and most of them, eventually, did not mate with a female (i.e. 'losers' N=19 out of 26)



**Fig. 3.** The sexual conflict: Males' mounting success in relation to the females' rejection attempts. Linear relationship of the sexual reciprocity during pre-copulatory interaction in gregarious locusts. **A-B.** No-choice interactions (N= 45): **A.** The pre-mounting stage (N= 45); **B.** The mounting stage (N= 44, 1 outlier). ● Mated locusts. ○ None mated locusts. **C-D.** Choice interactions (N= 74): **C.** The pre-mounting stage (N=68 out of 74, 6 showed no mounting attempt); **D.** The mounting stage (N=58 out of 74, 16 did not mount a female). ■ Mated locusts. □ None mated locusts. In **A & C:** x-axis – '*accumulated male failures*'. Y-axis: '*accumulated female rejections*' (walking or jumping away from the male). In **B & D:** x-axis - '*accumulated female rejections*' (by using direct rejection). Y-axis: '*accumulated male mounts*.' Numbers within the scatter symbols represent the number of interactions with similar values (superimposed). Outlier detection test, Mahalanobis Distance method. The dashed line represents a 95% confidence interval. A significant correlation was found in each of the four groups, Spearman's correlation test,  $p(v) < 0.05$ .

## Female's choosiness level

Testing for differences in choosiness levels (high, moderate or low) between the two treatments revealed a non-uniform distribution, with the majority of females displaying a high level of rejection (no-choice: ca. 67%,  $G_1^2=20.86$ ,  $p(v)<0.0001$ ; choice: ca. 70%,  $G_1^2=20.86$ ,  $p(v)<0.0001$ ) (**Fig. 4**). However, the number of available males did not significantly affect the choosiness level of females (two-sided Fisher exact test for multiple comparisons,  $p>0.05$ ).

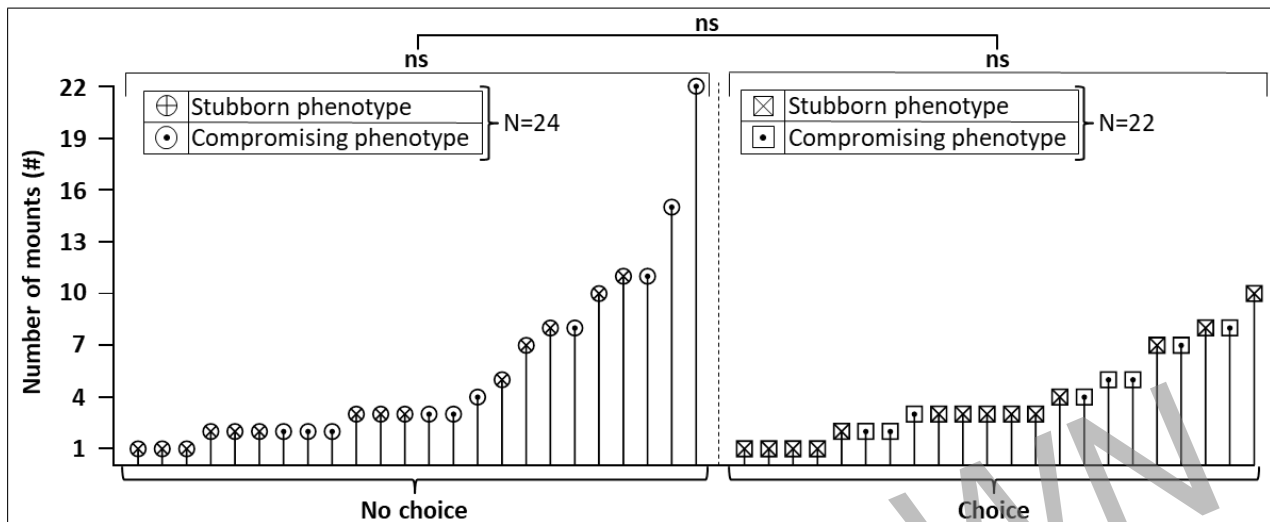


**Fig. 4.** Choosiness level of female locusts: High - rejection in the two pre-copulatory stages). Moderate - rejection in one pre-copulatory stage. Low - no rejection. Left – No-choice treatment (N=45 females); Right - Choice treatment (N=37 females). Within-group comparison (G-test of goodness of fit,  $DF=2$ ,  $p(v)<0.05$  in both groups). Between-groups comparison (Cochran–Armitage Chi-Square test for trend,  $DF=(1,5)$ ,  $p(v)>0.05$ ).

## Mounting stage as a major sexual conflict point

### Rejection threshold

All mated females that displayed a direct rejection against the mounting male were classified into two threshold phenotypes, *stubborn* and *compromising* (**Fig. 5**). In both treatments, the proportions of the two phenotypes were relatively similar (G-test of goodness of fit,  $p(v)>0.05$ ), with the *stubborn phenotype* being the dominant phenotype (No choice: N=24, *stubborn*– 58%, *compromising*– 42%; Choice: N=24, *Stubborn*– 64%, *compromising*– 36%). Also, the proportional distribution of the two phenotypes was similar in the two treatments (two-sided Cochran–Armitage Chi-Square test for trend,  $p(v)>0.05$ ).

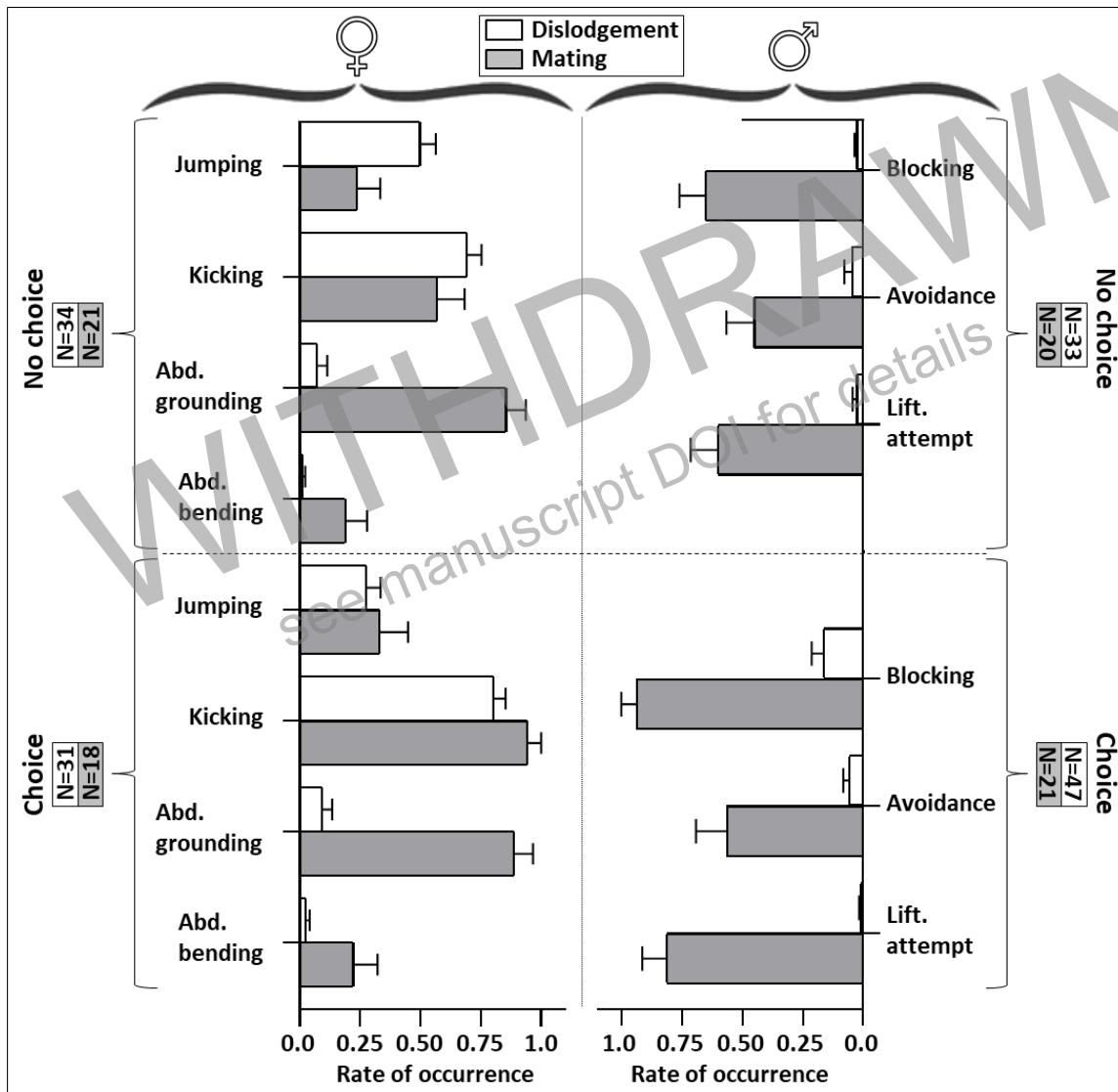


**Fig. 5.** Rejection phenotypes of mated females. Distribution of the two rejection phenotypes in all mated females that showed direct rejection during the mounting stage (no choice: N=22; choice: N=24). *Stubborn phenotype* – display of a direct rejection in all mounts (no choice: N=14 out of 24; choice: N=14 out of 22). *Compromising phenotype* – direct rejection was not performed (receptive) during the last mount that ended with mating (no choice: N=10 out of 24; choice: N=8 out of 22). For the within-comparison in each treatment, we used the G-test of goodness-of-fit. The two treatments were compared using the two-sided Cochran–Armitage Chi-Square test for trend.

### Antagonistic behavior

The behavioral repertoire strongly associated with sexual conflict was quantified (rate of occurrence using a Boolean scoring) among all interactions by which a female rejection was shown (direct rejection: jumping, kicking; indirect rejection: abdominal grounding, abdominal bending) (**Fig. 6**). A three-way repeated model (ART-ANOVA) of (1) the *treatment* (no-choice and choice), (2) the *outcome of each mount* (dislodgement or a mating), and (3) the *behavioral element* was conducted for each sex (**Supp. 1**). The three-way model revealed a significant effect on the rate of occurrence in both sexes (female:  $F_{15}=18.16$ ,  $p(v)<0.0001$ ; male:  $F_{11}=27.82$ ,  $p(v)<0.0001$ ). Further, in both sexes, the *outcome of each mount* and the *behavioral element* had a significant effect on the rate of occurrence (the *outcome of each mount*: Female,  $F_{1,56}=113.92$ ,  $p(v)<0.0001$ ; male,  $F_{1,48}=135.17$ ,  $p(v)<0.0001$ ) (the *behavioral element*:  $F_{1,168}=15.76$ ,  $p(v)<0.001$ ; male,  $F_{1,96}=19.05$ ,  $p(v)<0.001$ ). Among the female's behavioral elements, direct rejection by kicking was the dominant behavior, followed by jumping. These behavioral patterns were common in the two treatments, as shown by the insignificant effect of the *treatment* on the rate of occurrence ( $F_{1,56}=2.84$ ,  $p(v)>0.05$ , **Supp. 1**). The rate of occurrence of indirect rejection behaviors (i.e., abdominal grounding and abdominal bending) revealed significant differences between the two mount outcomes: a low rate of occurrence in mounts that ended in the male's dislodgement and a

high *rate of occurrence* in mounts that ended in mating (**Fig. 5, Supp. 1**). Among the male's behavior, the *rate of occurrence* of the three responding elements was significantly higher when the males' mounts ended with mating than when it ended with the males' dislodgement (See repeated-analysis in **Supp. 1**). *Treatment* has no significant effect on the *rate of occurrence* ( $F_{1,48}=4.84$ ,  $p(v)>0.05$ ).

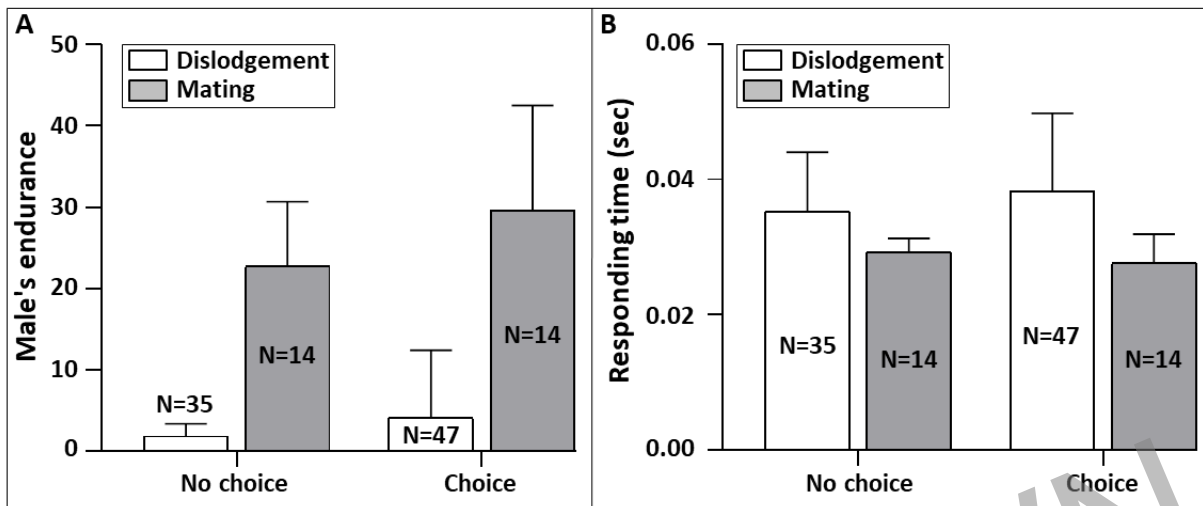


**Fig. 6. Antagonistic behavior** during the mounting stage. **Left** - *rate of occurrence* of the females' direct rejecting behaviors, jumping and kicking, and the indirect rejecting behavior, abdominal grounding, and abdominal bending. **Right** - *Rate of occurrence* of the male attempts to avoid the female rejection, blocking, avoidance, and lifting efforts of the female for a mating position. The conflict was tested in no-choice (females and males: N= 38 out of 45, 7 receptive females excluded from this analysis) and choice conditions (females: N=30 out of 35, 5 receptive females excluded from this analysis, males: N=53 out of 74, 5 males who mated with receptive female and 16 losers excluded from this analysis). The rate of occurrence of the behaviors was calculated for each of the two possible outcomes of a mount, *Dislodgement* (white) or *Mating* (gray). Statistical analysis

is presented in **Supp. 1**. Note that the two outcome groups of *dislodgement* were *mating* are not complementary, as they do not follow the assumption of independence.

### Male endurance

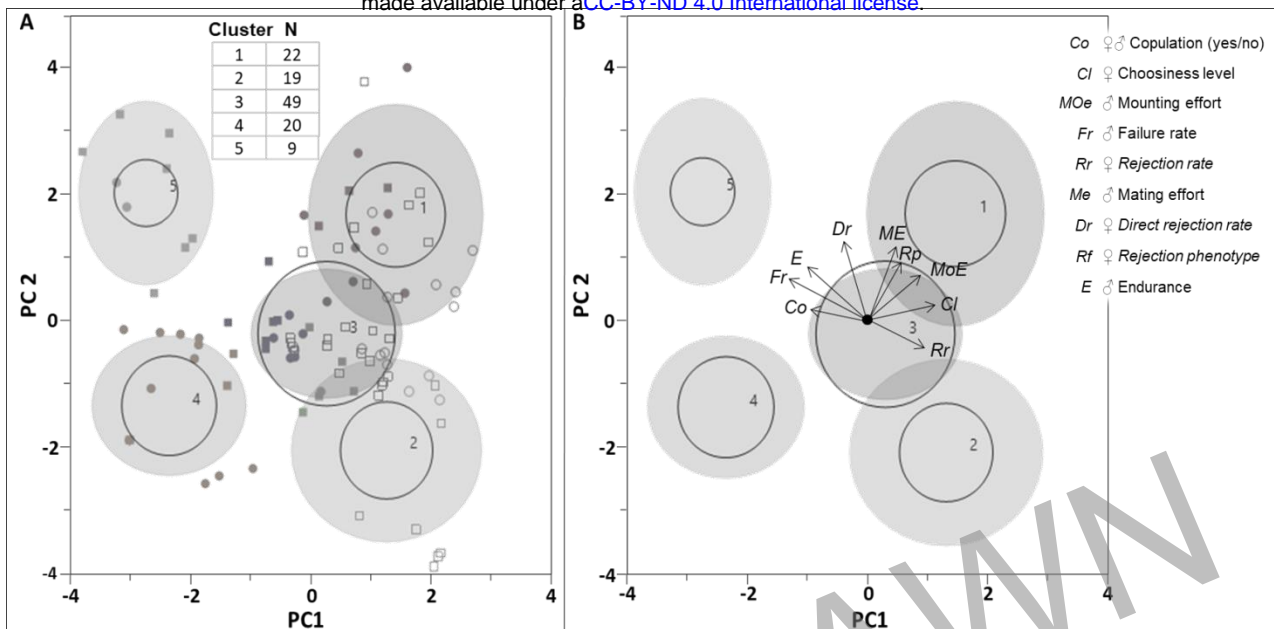
The ability of male locusts to overcome direct rejections of the females included two parameters (number of mounts with direct rejections: no choice, N=213; choice, N=202): (i) Male's endurance - the weighted average of the total frequencies of the female's jumping or kicking (**Fig. 7A**), and (ii) the responding time - the time (sec) from initiation of mounting by males until the female responded (**Fig. 7B**). Both parameters were tested using a two-way analysis of (1) *the treatment* (no-choice and choice) and (2) *the outcome of each mount* (dislodgement or a mating) (**Fig. 7**, for fully quantitative analysis see also **Supp. 2**). The results demonstrated a low endurance of males against the direct rejection behavior of females. For example, most of the males' mounts resulted in the dislodgement of the male (no choice: 182 dislodgements out of 213 mounts, ca. 85.4%; choice: 186 dislodgements out of 202 mounts, ca. 90.2%). Moreover, only a few males sustained the direct rejections and mated with a female without being dislodged (no choice: N=4; choice: N=6). Dislodgements of the males occurred after a short set of direct rejections (*dislodgement*: no choice, ca.  $1.8 \pm 0.27$  per mount; choice, ca.  $2.78 \pm 0.88$  per mount, **Fig. 7A**). On the contrary, mounts that resulted in mating were characterized by a pronouncedly higher ratio of direct rejections (*mating*: ca.  $20.54 \pm 27.19$  per mount, **Fig. 7A**). The two-way model (ART-ANOVA) revealed a significant difference between the two factors of *male endurance* ( $F_3=17.86$ ,  $p(v)<0.0001$ ). Further, the difference between the two categories of the *outcome of each mount* (dislodgement vs. mating) was significantly different ( $F_{1,17}=67.90$ ,  $p(v)>0.0001$ , **Supp. 2**). This tendency was found in both treatments (no choice and choice), as shown by the insignificant effect of *treatment* on the *male's endurance* ( $F_{1,17}=0.58$ ,  $p(v)>0.05$ , **Supp. 2**). Notably, in 16 out of the 27 interactions that ended in mating, 16 losers did not reach the mounting position. There was no significant effect of the treatment on the female's *responding time*, nor had the outcome of the mount (**Fig. 7B**) ( $F_3=0.8$ ,  $p(v)>0.05$ , **Supp. 2B**).



**Fig. 7.** Male's ability to overcome the female's direct rejection. **A**, Male's endurance against the female's direct rejection. X-axis: Treatments – no choice and choice. Y-axis: Weighted average of direct rejections per mount (mean + SEM). White columns – mounts that ended in dislodgement; Gray columns – mounts that ended in mating. **B**, time from initiation of the male mounting to the direct rejection displayed by females. X-axis: treatments. Y-axis: Responding time (mean + SEM). Color index and sample sizes are similar to the description in **Fig. 7A**. For full statistic analysis see **Supp. 2**.

*Sexual conflict at the population level*

In addition to examining the sexual conflict on the individual level, the intensity of the inter-sexual conflict was investigated at the population level. The clustering analysis results (**Fig. 8**) suggest that most interactions are characterized by an intense conflict expressed in strong female rejection during pre-mounting and mounting stages (clusters # 1-3, N=90 out of 119, ca. 76%). Cluster 2 is associated with non mating interactions, which comprise the sub-group of loss in mating due to intra-male competition (8 losers) that did not accomplish a successful mount. Notably, clusters 4 and 5 differed distinguishingly from other clusters. Cluster 4 is associated with calm conflict during pre-mounting but with an intense conflict during the mounting stage. Cluster 5 is associated with calm conflict during pre-mounting but high conflict during mounting.



**Fig. 8.** 2D-geometric landscape of the intensity of the sexual conflict in the gregarious locusts. The analysis was done by the K-Means clustering algorithm based on the following parameters: '*result of the interaction*' (mated or not), '*female choosiness level*', '*male mounting effort*', '*male failure rate*', '*female rejection rate*', '*male mating effort*', '*female rate of direct rejection*', '*female rejection phenotype*', '*male endurance*', and '*the outcome of the sexual interaction*'. Canonical cycles represent the domain area of each of the five centroids ( $k=5$ ). **A**, distribution of the 119 interactions in the first two principal components. **B**, degree of association of each parameter with each principal component. No choice: ● Mated and ○ none mated interactions. Choice: ■ Mated and □ none mated interactions.

### Discussion

In dense populations, the polyandrous mating system is often characterized by an overt female choice and intra-male-male competition (Arnqvist 1992; Härdling and Kaitala 2005; Arnqvist and Rowe 2013), thus intense sexual conflict is predicted (Parker 1983). The mating system of gregarious locusts presents a unique opportunity to measure the conflict expressed by a high local population density, a strong male-biased operational sex ratio, fierce scramble-competition among males for the access to females (Popov 1958; Uvarov 1977), and female strong rejection response (Golov et al. 2018a). Nevertheless, the current knowledge of the intra-sexual conflict in locusts is still limited (Golov et al. 2018a). This work aimed to close this gap by studying core aspects of the sexual conflict during the locusts' pre-copulatory interactions.

### Female mate choice

Mate preference is a central aspect of the sexual conflict (Parker 1983), characterized by the interplay between the effort invested in sampling potential mates and the cost involved in its continuous assessment (Thornhill 1980; Luttbeg 1996). When choosing a mate, female locusts face



the trade-off between high choosiness threshold and gaining a high-quality partner *versus* the increased costs associated with the mating attempts of competing males. Males, however, increase their mating success by ameliorating their endurance against the female's rejection efforts (Andersson 1994). In this study, gregarious female locusts revealed stereotypical rejection patterns when presented with one male or a choice of two males. This finding supports previous studies describing the locusts' sexual behavior as highly conservative and phase-dependent (Golov et al. a,b). Sexual selection promotes female choosiness in high-density polyandrous mating systems (Wickman & Rutowski 1999). In accordance, this study demonstrates that the vast majority of gregarious females were highly choosy, strongly rejecting the males' mating attempts. A similar increase in the female's choosiness at high densities has been shown for pied flycatchers (*Ficedula hypoleuca*) (Alatolo et al. 1988a), the katydid (*Kawanaphila nartee*) (Shelly & Bailey 1992) and kestrels (*Falco tinnunculus*) (Palokangas et al. 1992) (see review Jennions & Petrie 1997).

Females' sampling strategy is central to mate assessment (Janetos 1980). In locusts, unlike many other grasshoppers, males do not provide mating nuptial gifts (Otte 1970) or other resources that may facilitate females to assess the male's quality (Borgia 1979). Furthermore, during the pre-copulatory interaction, gravid females experience simultaneous harassment (scramble competition) by the many males that attempt mating (Popov 1958; Strong & Amerasinghe 1977). Under these crowded conditions, females should not adopt sampling strategies that require returning to a specific male, such as "*best-of-N*" or "*sequential-comparison*" (Janetos 1980). The females' sampling method, known as "*Random sampling*" (Real 1990), should also be excluded; as was revealed in this study, most of the females did not mate with the first encountered candidate. This study suggests that gregarious female locusts stereotypically reject males' mating attempts. Moreover, a considerable number of rejecting females (ca. 40%) have relaxed their stubborn response through time. Therefore, we deduced that gregarious females use the '*adjustable internal acceptance threshold*' (Reid & Stamps 1997). Specifically, the female's pre-mating behavior best suits the '*one step decision rule*' strategy (or '*sequential search rule*', Janetos 1980; Real 1990), setting a certain threshold for male quality in the pre-mounting stage and a different threshold for males that passed this first test and have reached the mounting stage. The acceptance threshold of the two stages has been probably reset constantly. The optimal threshold may be derived from the interplay between the cost of continuous searching and the probability of finding a better mate. A similar dynamic acceptance threshold has been demonstrated for choosy gregarious females, as some of them accepted a mate right after dislodging another potential mate (by lowering their acceptance threshold, i.e. '*Compromising phenotype*'), while other females maintain their high acceptance threshold until mating (i.e. '*Stubborn phenotype*').

The high acceptance threshold (high choosiness level) showed by the gregarious females in the pre-mounting stage fits the theoretical models of highly dense promiscuous mating systems (Parker 2006; Perry & Row 2015). Notably, the two pre-copulatory stages differ in relation to the mechanism of mate assessment. A better evaluation of potential mates can be executed during the mounting stage when physical contact between the sexes takes place. This understanding is in line with the '*the screening hypothesis*' proposed by Arnqvist and Rowe (2005), which holds that a female selects her mate based on his ability to tolerate her rejection. Similar rejection behavior of females was reported in other dense promiscuous mating systems of insects, where prior information of potential mates is unavailable (field crickets, Souroukis and Murray 1994; water strider, Arnqvist 1995; *Drosophila melanogaster*, Chapman & Partridge 1996; seaweed fly, Weall and Gilburn 2000; yellow dung fly, Blanckenhorn et al. 2000; bean weevil, Maklakov et al. 2005). Hence, gregarious female locusts may apply a *screening* mechanism in a '*one step decision rule*' to discriminate among potential mates under conditions of a fierce intra-male competition (e.g. swarming conditions). We suggest that the observed sampling strategy in female locusts is conservative, as the number of potential mates had no clear effect on the distribution of the rejection *phenotypes* among females.

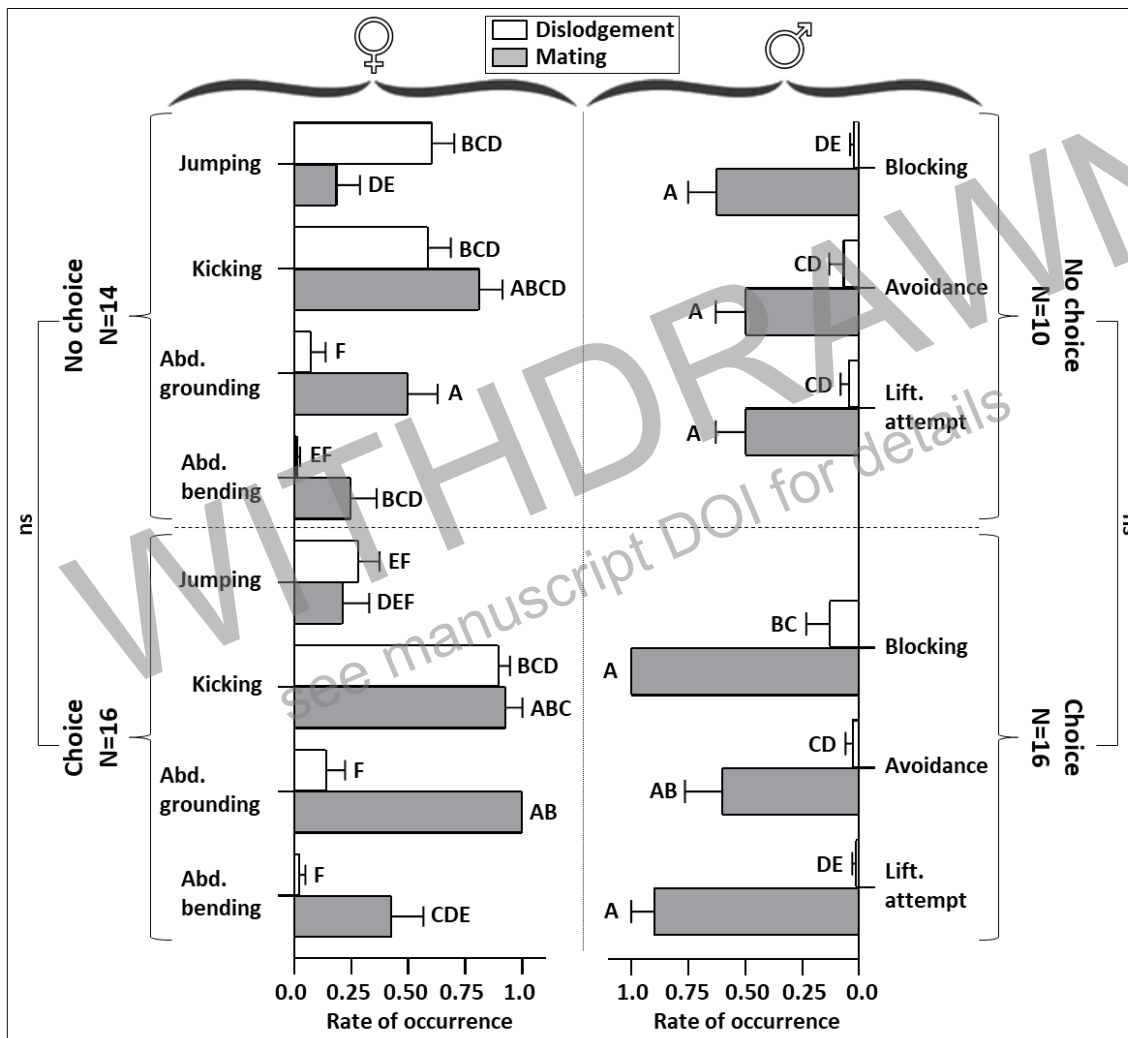
From the male point of view of the sexual conflict, the tug-of-war of counter-adaptations between the sexes has led to the adaption of traits that allow overruling the female rejection efforts (Arnqvist and Row 2002). Our results provide evidence for such adaptive male mating behaviors: (i) Sneaking ability - reaching the female unnoticed and avoiding her sliding away from the approaching male. This behavior ('sneak-mating strategy') is common among males in mating systems having high intra-male competition ('sneaking fertilizations', Parker 1998). (ii) Mounting precision – the male's accurate mounting on the female back provides an advantage due to the jumping response of the female and the co-occurrence of mounting attempts of rival males. (iii) Endurance – the male's ability to withstand direct rejections of the females. Endurance was suggested as an important characteristic of successful males in the promiscuity mating system (Thornhill & Alcock, 1983; Crean & Gilburn 1998). The responding time of females was very fast ( $\bar{x}$ =ca. 0.14 sec), leaving a concise time window for the male to set his position for an optimal grasp of the female. The majority of males that experienced direct rejections were dislodged from the females at least once. Successful mounts that ended with mating were characterized by a high frequency of direct rejection, nevertheless, evidence for the effect of size on the male mating success was not detected. This suggests that the ability to endure direct rejection, *perese*, serves as a trait of female preference in the gregarious locusts (following the theory of 'sexy sons effect', Fisher 1930). This suggestion is supported by evidence of our previous work, showing that constraint females (with tethered hind legs) had no ability to reject males, and eventually all were mated (Golov et al. 2018a). The grasping behavior has been suggested as a trait under sexual

selection in other insects such as the water strider (Arnqvist 1995), diving beetle (Bergsten et al. 2001), and the seaweed fly (Crean & Gilburn 1998). However, unlike water striders and diving beetles, the desert locusts (males and females) lack the antagonistic 'grasping-antigrasping' morphological armaments. Hence, we suggest that the male locusts' defensive behavior (i.e., *blocking* and *avoidance*) against a direct female rejection has evolved as a counter-adaptation to the direct rejection behavior of the female. It is worth mentioning that locusts' postcopulatory behavior is characterized by male mate-guarding due to the last male's sperm precedence (Dushimirimana et al. 2015). This emphasized the high selection pressure that operates on gregarious males due to both choosy females and a strong intra-male rivalry.

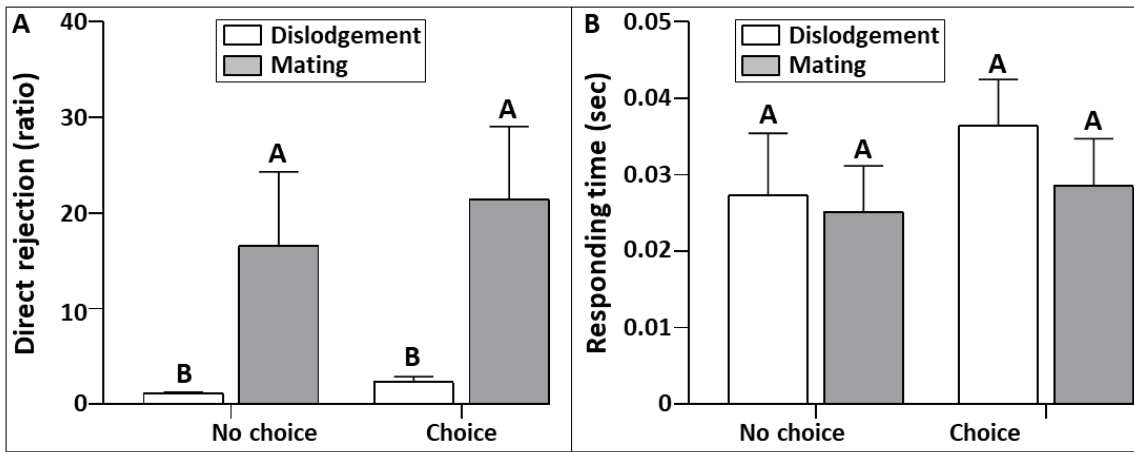
The ecological conditions and the selection forces ('eco-evolutionary dynamics') concomitantly contribute to shaping the mating system (Hendry 2016) and to the dynamics of the sexual conflict (Svensson 2019). Yet, data of the effects of eco-evolutionary dynamics on sexual conflicts in ecological contexts (ecologically dependent selection) are limited (Arbuthnott et al. 2014; Perry & Row 2015). As regards to that, mating system of locusts may serve as a good eco-evolutionary model in understanding the modification mating system. The gregarious locust female-predominated mate choice strategy was also found in other mating systems in similar ecological conditions (kestrels, Palokangas et al. 1992; katydids, Shelly & Bailey, 1992; water striders, Arnqvist 1995; seaweed fly, Crean & Gilburn 1998; fireflies, Cratsley and Lewis 2005). Future study of locusts polyphenism in the context of sexual selection will further contribute to the understanding the of eco-evolutionary dynamics of sexual conflicts.

Supplementary

In order to provide comprehensive analyses for the *Antagonistic behavior* (Fig. 6) and for *Male endurance* (Fig. 7), a quantitative analysis of three-way repeated model of (ART-ANOVA) of the '*rate of occurrence*' has been done for each sex independently (descriptive statistic in Fig. 6). A two-way repeated test (ART-ANOVA) was done for the parameters '*Male's endurance*' (descriptive statistic in Fig. 7A), and the '*responding time*' (descriptive statistic in Fig. 7B).



**Supp. 1.** Repeated analysis of the rate of occurrence of the behavioral elements that are related to sexual selection during the mounting stage. Description is similar to that given in Fig. 6, yet the data used here follows the assumption of dependency (see 'Two-way criterial procedure' in 'Material & methods'). Females: No choice,  $N_{\text{Dislodgement}} = N_{\text{Mated}} = 16$ , Choice,  $N_{\text{Dislodgement}} = N_{\text{Mated}} = 14$ . Males: No choice,  $N_{\text{Dislodgement}} = n_{\text{Mated}} = 10$ , Choice,  $N_{\text{Dislodgement}} = N_{\text{Mated}} = 16$ .



**Supp. 2.** Males endurance against female's direct rejection. Description is similar to that given in **Fig. 7**, yet the data used here follows the assumption of dependency (see 'Two-way criterial procedure' in 'Material & methods'). Locusts (females or males): No choice,  $N_{\text{Dislodgement}} = N_{\text{Mated}} = 11$ , Choice,  $N_{\text{Dislodgement}} = N_{\text{Mated}} = 16$ .

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