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Precopulatory behavior and sexual conflict in the desert locust

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Running title: Desert locust sexual behavior

20 **Abstract**

21 Studies of mating and reproductive behavior have contributed much to our understanding of various
22 animals' ecological success. The desert locust, *Schistocerca gregaria*, is an important agricultural pest.
23 However, knowledge of locust courtship and precopulatory behavior is surprisingly limited. Here we
24 provide a comprehensive study of the precopulatory behavior of both sexes of the desert locust in the
25 gregarious phase, with particular emphasis on the conflict between the sexes. Detailed HD-video
26 monitoring of courtship and mating of 20 locust pairs, in a controlled environment, enabled both
27 qualitative and quantitative descriptions of the behavior. A comprehensive list of behavioral elements
28 was used to generate an eight-step ethogram, depicting from first encounter between the sexes to
29 actual copulation. Further analyses included the probability of each element occurring, and a kinematic
30 diagram based on a transitional matrix. Eleven novel behavioral elements are described in this study,
31 and two potential points of conflict between the sexes are identified. Locust sexual interaction was
32 characterized by the dominance of the males during the pre-mounting stage, and an overall stereotypic
33 male courtship behavior. In contrast, females displayed no clear courtship-related behavior and an
34 overall less organized behavioral sequence. Central elements in the sexual behavior of the females
35 were low-amplitude hind-leg vibration, as well as rejecting males by jumping and kicking. Intricate
36 reciprocal interactions between the sexes were evident mostly at the mounting stage. The reported
37 findings contribute important insights to our knowledge of locust mating and reproductive behavior, and
38 may assist in confronting this devastating agricultural pest.

39

40

41 **Key words:** *Schistocerca gregaria*, courtship, sexual interaction, mounting, rejection

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43

44 **Introduction**

45 The desert locust, *Schistocerca gregaria*, is one of the most serious agricultural pests. During
46 outbreaks, swarms may consist in millions of individuals (40-80 million locusts per km²; e.g. Singh &
47 Singh 1977; Steedman 1988; El Bashir et al. 1993), and the damage to crops can be enormous, as the
48 locusts are able to consume hundreds of tons of vegetation per day (Shaluf 2007). Moreover,
49 according to some estimates, 1/10 of the global human population is affected by this pest (Latchininsky
50 et al. 2011).

51 Locusts have served as important models in the study of various aspects of insect physiology and
52 behavior (e.g. Burrows 1996; Ayali & Yerushalmi 2010; Ayali & Lange 2010; Ariel & Ayali 2015).
53 However, our knowledge of locust courtship and precopulatory behavior is surprisingly limited.
54 Understanding the mating and reproductive behaviors of a species has a fundamental role in the
55 understanding of its ecological adaptation (Kirkendall 1983; Thornhill & Alcock 1983). Specifically,
56 thorough comprehension of the interactions between the sexes may provide new insights for the
57 development of alternative methods for coping with agricultural pests (Boake et al. 1996; Suckling
58 2000). This should be achieved by a combination of qualitative descriptions and quantitative analyses-
59 the two complementary components of an ethological study (Kasuya 1983).

60 A comprehensive study of animal behavior should start with a list of behavioral elements (or 'units'),
61 followed by their chronological appearance, in order to construct a species-specific **ethogram**. The
62 quantification of behavioral elements needs to be based not only on the appearance of these elements,
63 but also on their frequency, their sequence and the probability of transition. Such an approach can
64 identify the typical elements and key transitions during the behavioral ritual (e.g. Klein & De Araújo
65 2010). The quantification can be aided by using a Markovian chain, also known as a transition matrix
66 (Castrovillo and Cardé 1980; Haynes and Birch 1984). The knowledge gained may contribute not only
67 to deciphering evolutionary relationships between taxa, as in host-parasite interactions, but also to the
68 understanding of mate recognition and sexual conflicts (Paranjape 1985; Curkovic et al. 2006; Cozzie &

69 Irby 2010; Gaertner et al. 2015), and specifically so in agricultural pests (Walgenbach & Burkholder
70 1987; Rojas et al. 1990; Wang & Millar 2000; Zahn et al. 2008).

71 Some aspects of the sexual behavior of *S. gregaria* have been previously addressed (Uvarov 1928;
72 Husain and Mathur 1946; Laub-Drost 1959, 1960 cited in Uvarov 1966, 1977; Popov 1958; Loher 1959,
73 1961; Pener 1965, 1967a,b; Norris 1964; Odhiambo 1966; Roffey & Popov 1968; Strong &
74 Amerasinghe 1977; Uvarov 1977; Amerasinghe 1978a; Pener & Lazarovici 1979; Inayatullah et al.
75 1994; Njagi & Torto 2002), but much of the required knowledge is still lacking. The published
76 descriptions and quantifications of the sexual behavior of both sexes are either limited (Strong &
77 Amerasinghe 1977; Inayatullah et al. 1994), too general, or focus predominantly on the male (e.g Pener
78 1967b; Amerasinghe 1978b). In addition, previous studies suffer from inconsistencies (e.g. different
79 names for similar behavioral element). Finally, little effort has been dedicated to the study of sexual
80 conflict in this insect. The desert locust displays a clear sexual dimorphism in the gregarious phase,
81 with fully mature males being bright yellow and females being beige-brown to yellowish (Chauvin 1941
82 cited in Pener & Simpson 2009; Norris 1954; Pener 1967b). As is the case for many other Acridids,
83 little is known regarding the means of sexual recognition in the desert locust (Whitman 1990). It is
84 postulated, however, that visual and chemical signals play an important role (Obeng-Ofori et al. 1993,
85 1994; Franck and Schmidt 1994; Inayatullah et al. 1994; Ely et al. 2006; Seidelmann & Warnstorff
86 2001). Courtship and mating behaviors can be roughly divided into two sequential stages: pre-
87 copulatory and post-copulatory (with copulation defined as the time when sperm is transferred). The
88 pre-copulatory stage can be divided into two further sub-stages: pre-mounting, comprising all the
89 behavioral elements leading to a successful mounting attempt; and mounting, culminating in successful
90 copulation. Locust courtship is considered simple and primitive (Loher 1959; Uvarov 1966, 1977;
91 Oberlin 1974 cited in Strong & Amerasinghe 1977). As in many grasshoppers, males of *S. gregaria*
92 have been reported to be the dominant gender during the sexual-interactions (Norris 1964; Pener 1965,
93 1967b; Strong & Amerasinghe 1977; Amerasinghe 1978a; Inayatullah et al. 1994). Briefly, the male's

94 sexual intention is initially demonstrated through his orientation towards the female, followed by a
95 stealthy slow approach and a surprise attempt to mount her. Once mounting, the male grasps the
96 female using his front and mid-legs. Copulation is achieved when the male moves his abdomen along
97 the side of the female and connection between the genitalia is established. In contrast to the males,
98 gregarious females have been considered to demonstrate no clear courtship behavior (Norris 1964;
99 Pener 1965, 1967b; Strong & Amerasinghe 1977; Amerasinghe 1978a; Inayatullah et al. 1994).
100 Nonetheless, the rejection of courting males has been reported, including the female's jumping (before
101 and during mounting), kicking, and lateral movements of her abdomen in the attempt to prevent
102 copulation (Loher 1959; Strong & Amerasinghe 1977; Uvarov 1977). Hind leg vibration and wing
103 stridulation have been reported to be displayed during the pre-copulatory behavior (Morse 1896; Norris
104 1954; Laub-Drost 1959 cited in Uvarov 1977; Loher 1959, 1961; Otte 1970; Uvarov 1966, 1977), as in
105 other acridid grasshoppers (Haskell 1957, 1958; Otte 1977). Unlike wing stridulation (displayed by both
106 sexes), the vibration of the hind legs is soundless and much more common in the female (Loher 1959).
107 The role of both behavioral elements in the sexual interaction has remained uncertain (Loher 1959;
108 Uvarov 1966; Otte 1970).

109 The major goals of this work were to generate an ethogram, comprising and accompanied by both
110 qualitative and quantitative tools for studying the sexual behavior of the two sexes of the desert locust
111 during the pre-copulatory stage. This included generating a detailed list of all related behavioral
112 elements, and consolidating the relevant terminology (i.e. '*nomenclatura*'). The generated ethogram
113 includes all the behavioral elements, their occurrences, and their sequence during the sexual
114 interaction. This had enabled an elaborate description of the conflict between the sexes in gregarious
115 locusts. In an accompanying study (Golov et al., in preparation), we employ the tools developed herein
116 for a comparative investigation of the two density-dependent locust phases.

117

118

119 **Material & Methods**

120 *Animals*

121 Desert locusts, *Schistocerca gregaria*, from our colony at Tel Aviv University (Ayali et al., 2002) were
122 reared for many consecutive generations under crowded conditions (i.e. approaching the gregarious
123 phase), 100-160 individuals in 60 aluminum cages. All cages were located in a dedicated room under a
124 constant temperature (29-31°C) and light cycle of 12: 12 D: L. Supplementary radiant heat was
125 supplied during day-time by incandescent 25 W electric bulbs, resulting in a day temperature of c.
126 37°C. Locusts were provided daily with fresh wheat and dry oats, and plastic caps (300cc) filled with
127 moist sand for oviposition.

128 All locust individuals in the experiments were adult virgin males and females. Virgin adults were
129 obtained by marking newly-emerged adults with non-poisonous acrylic paint within 24 hours following
130 ecdysis. Males and females were separated into single sex “cohort cages” every 3 days. Thus, in each
131 cohort cage the maximum age range of the individual locusts was less than 72 hours. The cages were
132 maintained under the same rearing conditions as above. For the observations we used 12-14-days-old
133 males, when their yellowish coloration had reached stage V (see Norris 1954 & Loher 1961). This stage
134 is known to coincide with sexual maturity. Females were 18-20 day-old, sexually mature, based on our
135 preliminary work and other previous reports (Hamilton 1955; Injeyan & Tobe 1981; Mahamat et al.
136 1993; Wybrandt & Andersen 2001; Ould Ely et al. 2006; Nishide and Tanaka 2012). Only fully intact
137 insects participated in the observations.

138 *Experimental design*

139 Experiments were carried out in an isolated room, with temperature and light conditions similar to that
140 in the rearing room. A plastic observation cell (14x13x24 cm) was initially divided, by an opaque plastic
141 partition into two compartments, to separately host the male and the female. The sensitivity of *S.*
142 *gregaria* color vision is mainly in the very short wavelengths of both UV and blue (320 and 450-nm),
143 and to a lesser extent, also in the green range (light 530-nm) (Eggers & Gewecke 1993; Schmeling et

144 al. 2014). Hence, until initiation of the experiment the cell was illuminated by a red light (to reduce the
145 insects' stress). Five minute after placing each locust (one male and one female) into its own
146 compartment, the experiment was initiated by carefully removing the partition between the
147 compartments, and replacing the red light with two regular 25 W light bulbs. Two identical observation
148 cells, separated only by a dens plastic mesh (not sealed), were used simultaneously (each housing one
149 pair of locusts), generating crowd-like conditions by allowing the flow of auditory, olfactory and visual
150 cues. Experiments lasted 3 hours, or until copulation had occurred, if earlier, and were recorded by a
151 SONY HDR-PJ820E video camera.

152 Two rounds of experiments were carried out daily: at 08:00 AM and 15:00 PM. Out of an overall 31
153 monitored experiments, 20 ended in copulation within the defined time of 3 hours, and were used in the
154 analyses.

155 In order to further verify the significance of the females' active rejection behaviors (jumping and kicking)
156 and their tentative role in female choice, a separate series of experiments were carried out. Here we
157 examined male mating success when facing "handicapped" females. The rejection attempts of these
158 females were constrained by means of a small rubber band confining the hind legs' femur and tibia in a
159 folded position, and thus, preventing the female from either jumping or kicking. The number of male
160 mounting attempts and successful mounts were compared between pairs of males and
161 constrained females (N=10) and males and unrestrained females (N=20).

162 *Data analyses*

163 The recorded videos of the behavior of each pair were reviewed and analyzed using J-watcher software
164 (version 0.9 for Windows).

165 Behavioral elements were identified in order to construct the locusts' pre-copulatory behavior. These
166 included both repetitive (lengthy, e.g. the vibration of the hind leg femur) and discrete (momentary, e.g.
167 jumping) behaviors. The two behavioral types were counted, with a 'count' relating to the duration of a

168 behavior from initiation until termination. Behavioral measurements were taken only if the male and
169 female were at a distance of less than 10 cm (i.e. an 'encounter'). For both pre-mounting and mounting
170 behaviors, the following parameters were measured and compared for both sexes: (1) In order to obtain
171 the pattern or chronological sequence of the behavioral repertoire, the relative time to initiation of each
172 behavior was noted (relative to the total time of the relevant stage, either pre-mounting or mounting).
173 (2) The probability of a specific behavior occurring (PO=1 if the behavior occurred at least once, and 0
174 otherwise). (3) The frequency of occurrences of a specific behavioral element.

175 A kinematic diagram was constructed, based on a first-order Markov model, for all the transitions
176 between pairs of behavioral elements (i.e. preceding—following elements) that are mutually exclusive
177 (Baker & Cardé 1979). All the behavioral elements in this analysis were considered nodes and used to
178 construct a transitional matrix. The transition probability (TP, also known as 'conditional probabilities';
179 Wood et al. 1980) was first calculated based on all possible transitions between a pair of nodes in the
180 matrix, for each experiment (see also Brown 1974; Leonard and Ringo 1978; Markow and Hanson
181 1981). Next, the average of each transition was calculated among all 20 pairs for each sex (following
182 the method described by Charlton & Cardé 1990). Self-transitions were scored as structural zeroes
183 (Baker & Cardé 1979), and impossible transitions were left blank (Haynes & Birch 1984). Those
184 behavioral elements that were not mutually exclusive with any of the other elements ('antennal
185 movement', 'palp vibration', 'genital-opening', 'abdominal wagging') were excluded from this analysis.
186 Thus, the behavioral transitional matrix comprised 25 elements for the male, and 18 for the female.
187 Transitional probabilities (i.e. TP) $\leq 10\%$ are not presented.

188 Most of the statistical output and data analysis were conducted in GraphPad Prism version 6.04 for
189 Windows, JMP®, Version 12.0.1 SAS Institute, and some in Matlab (MathWorks, USA Inc.) and Canvas
190 draw 2.0 (Deneba Systems, Miami, FL).

191

192 **Results**

193 *The sexual behavior of the desert locust*

194 As noted above, the behavioral elements that lead to copulation (i.e. those that can be identified during
195 the pre-copulatory phase), can be divided into two stages: Table 1 lists all the elements comprising the
196 pre-mounting stage, and Table 2 lists all the elements comprising the mounting stage (ending in
197 copulation). Among the behavioral repertoire listed in Table 1 and 2, several elements have been
198 described previously. However, those descriptions tend to be episodic, with different authors providing
199 different descriptions for the same behavior, or referring to the same behavior by different names, etc.
200 Eleven elements are novel, and are described here for the first time.

201 The probability of each element being demonstrated varies greatly. Figure 1 denote the probability of a
202 behavioral element occurring (PO), separately for males and females for the pre-mounting and mounting
203 stages. The behavioral elements appear in a consecutive order and are grouped following a further
204 subdivision: S1-S7, from initiation (S1) to copulation attempt (S7), culminating in S8, copulation. Figure
205 1 presents the elements that involve all body parts (denoted by different colors), including legs, wings,
206 palps and antenna, and abdomen. Some of the sub-stages are characterized by a consistently high PO
207 (e.g. S1 during the pre-mounting; Fig. 1) while that of others varies greatly. Moreover, the PO of the
208 elements demonstrated by the male or the female within the same sub-stage differs (e.g. compare S1-2
209 or S5-7 in Fig. 1). Generally speaking, a high PO reflects the importance of a behavioral element within
210 the overall sequence. However, there may be low PO elements that nonetheless have a crucial
211 functional significance: e.g. those instrumental in inter- and probably also intra- sexual communication
212 (e.g. leg vibration, wing flutter and stridulation). Illustrations of the different behavioral elements are
213 provided in figure 2.

214 An ethogram was constructed (Fig. 3) in order to better characterize the behavioral sequence
215 comprising the pre-copulatory behavior. The ethogram provides the pre-mounting and mounting stages
216 (consistent with Fig. 1), presenting them as an ordered, hierarchical flow-chart. This representation also

217 allowed us to include and emphasize junctions or decision points (denoted by the traffic lights in Fig. 3).
218 These junctions represent the culmination of the conflict between the sexes, e.g. a point at which the
219 female was successful in preventing a mounting attempt by jumping away, or a point at which the male
220 was thrown off the female's back. Illustrations of behavioral elements of an antagonistic nature can be
221 seen in Figure 4.

222 Further information regarding the flow of the behavioral elements and the overall sequence of the
223 behavior can be obtained by also including, beyond the ordered description of the elements, the
224 probability of a transition from one element to the other. This approach regards the behavioral
225 sequence as a Markov process or Markov chain, in which the appearance of each behavioral element
226 affects or predicts the probability of the appearance of another. Figures 5 and 6 use a similar color code
227 as that presented in Figure 1 to indicate the different behavioral elements constituting the sub-stages
228 (S1-8), presented in Figures 1 and 3. These kinematic diagrams denote a weighted directed network
229 composed of the above introduced different behavioral elements presented by males (Fig. 5) and
230 females (Fig. 6), where the weights are the transition probabilities (TP). As can be seen, this method of
231 presentation clearly discriminates between behavioral elements constituting the relatively consistent or
232 major trunk (depicted 0-8 in Fig. 5, and 0-5 in Fig. 6), as well as the various possible detours or
233 diversifications from it. It also serves to highlight several sex-specific characteristics, as discussed
234 below.

235 In the following we provide further details of certain male- and female-specific behavioral elements, as
236 well as further insights into the conflict between the sexes.

237 *Sex-specific sexual behaviors and conflict between the sexes*

238 The strategy employed by males during pre-mounting can be described as stalking, pursuit and attack.
239 Overall courtship in our experiments was somewhat limited. Upon identifying the female, the male
240 commonly demonstrated 'high-stepping walking' behavior, carrying his body high above the ground. In

241 some cases, this was intensified prior to jumping in an attempt to mount the female, to an extent that
242 his front legs were raised in the air. Increased self-grooming was shown by all males (PO=100%);
243 males groomed the antennae, the compound eyes, the front or mid pairs of legs and the posterior part
244 of the abdomen. Several behavioral elements, commonly shown during pre-mounting, are described
245 here for the first time. These comprise: lateral wagging movements of the abdomen ('abdominal
246 wagging'), repeated extension movements of the subgenital-plate and the epiproct ('genital-opening'),
247 and repeated slow elevation of the hind legs. The latter was performed by most males (PO=95±5%)
248 just after (TP=14%) or before (TP=11%) approaching the female.

249 Once successful in mounting the female's back, mostly via jumping, the majority of males were quick to
250 cling to the lateral sides of her pronotum socket (or its edges; see Fig. 2D and E) in order to adjust their
251 grip. Stridulation and hind leg vibrations were more frequent during mounting than pre-mounting,
252 although the cumulative time of mounting (1.65 ± 0.41 min) was much shorter than in pre-mounting
253 (64.23 ± 10.93 min).

254 The females' overall sequence of behavioral elements was much less stereotypic compared to that of
255 the males (as also evident from Figs 1, 5 and 6). In spite of the dominant part played by males, the first
256 indication of encounter was usually demonstrated by females (17 out of 20 pairs). Hind leg vibration
257 was a characteristic element of females pre-copulatory behavior, as demonstrated by the high values of
258 both PO and TP (Fig 1 and 6). However the most dominant behavioral feature was the female rejection
259 of the males (Fig. 4).

260 During the pre-mounting stage, female rejection was displayed by either jumping or walking away from
261 the male. 'Walking away' (PO 75%) was commonly followed by the lower amplitude hind leg vibration
262 (TP=24%). The most common rejection element during the mounting stage was kicking (PO=55%).
263 Both kicking and jumping often caused the mounted male to lose his grip and dislodge from the females
264 back. In fact, more than half of the mated males were dislodged from the female (PO=55%). Females

265 also exhibited "passive rejection" elements, including pressing the abdomen against the ground and
266 thus preventing the male from inserting his abdomen below hers ('abdominal grounding' described here
267 for the first time; Fig 4C) and less frequently lateral abdominal bending (Fig 4B; PO=5%), which was
268 very efficient in preventing copulation.

269 Male behavioral elements that were intended to avoid or overcome female rejection are also described
270 here for the first time. These comprised: attempting to block the female's kicks with the male's own hind
271 legs (Fig 4A), and elevation of the hind legs with the tibia extended, while keeping the legs close
272 together, in order to minimize the area exposed to the female's kicking (avoidance; Fig 4B). Naturally,
273 males occupied with these defensive behaviors could not progress toward copulation. An intriguing
274 newly described element is that of the male's attempt to overcome the female abdominal grounding
275 behavior by pushing with his hind legs and lifting her up (Fig 4D). This reciprocal interaction is shown in
276 a specific path of transitions in Figure 5- 'mounting' → 'grounding of the hind legs' → 'lifting attempt'
277 → 'copulation attempt'.

278 In order to further explore the selected "female choice" stages (S3 and 5; Fig. 3) and verify the
279 significance of female active rejection behaviors and their effect on the males' mating success,
280 we prevented females from jumping and kicking by means of a small rubber band over their
281 folded hind legs. This manipulation indeed resulted in no active rejection by the constrained
282 females. Consequently, the number of male mounting attempts on these females was
283 significantly lower than that in the control group (med=1<2, U= 40.50, N₁=10, N₂=20; p<0.01),
284 and 100% of the pairs comprising a constrained female and a normal male ended in
285 copulation. The males that mounted constrained females displayed none of the documented
286 defense behaviors (see above).

287

288 **Discussion**

289

290 The sexual behavior of the desert locust *S. gregaria* has been previously addressed in various studies
291 (e.g. Uvarov 1928, 1977; Norris 1964; Pener 1965; Odhiambo 1966; Roffey & Popov 1968;
292 Amerasinghe 1978a; Pener & Lazarovici 1979; Njagi & Torto 2002), and was mostly described as
293 primitive and reduced (Popov 1958; Loher 1959; Strong & Amerasinghe 1978b; Pener & Shalom 1987;
294 Inatullah et al. 1994). None of those studies, however, were dedicated to a synchronized,
295 comprehensive investigation of the behavior of the two sexes and their sexual interactions. Loher
296 (1959) for example, although devoting much effort to describing locust sexual behavior, did not include
297 any quantitative measures of the different behavioral elements. In a first attempt to quantify the pre-
298 copulatory behavior of the male, Pener (1967a,b) employed a measure of “average percentage of time
299 spent on sexual behavior”, but with sexual behavior comprising only copulation, sexual attack, or
300 mounting another locust. In a later report, recording the time spent in sexual behavior, Wajc and
301 Pener (1969) noted the great need for elaborate quantitative methods in the study of the sexual
302 behavior of *S. gregaria*. While other quantification efforts (e.g. Inatullah et al. 1994) presented some
303 accounts of behavioral elements, they provided only limited descriptions of the pre-copulatory behavior
304 in a rather anecdotal manner, and similar to previous work focused mostly on males.

305 In the current study we provide in-depth data on the pre-copulatory behavior of the desert locust in the
306 gregarious phase, comprising both qualitative descriptions and quantitative measures. A detailed list of
307 behavioral elements is presented, incorporating eleven elements that are described here for the first
308 time. An ethogram of the sexual behavior of both sexes, from first encounter until copulation, has
309 enabled us to describe the dynamics of the behavior, including the probability of each element being
310 demonstrated and the transitions between elements. Overall eight distinct steps were identified as
311 comprising the two pre-copulatory stages: pre-mounting (S1-3) and mounting (S4-8). Most importantly,
312 two points of conflict between the sexes were recognized and investigated in depth.

313

314 *Male sexual behavior*

315 A major characteristic of locust sexual behavior is that of the males' dominant role in the courtship ritual
316 (Norris 1964; Strong & Amerasinghe 1977; Inayatullah et al. 1994). Our findings well demonstrate this
317 point: that the overall initiative is always by the male. Upon encountering a female, the males displayed
318 a combination of self-grooming, palp vibration and antennal movements (see also Loher 1959). The
319 latter is a known characteristic of male sexual behavior in the family Acrididae (Pickford & Gillott 1972;
320 Otte 1970; Riede 1987). Onset of the rather limited courtship behavior can be recognized initially by the
321 display of "orienting", in which the male points his antennae towards the female. This behavior is
322 common in the subfamily Catantopinae (Otte 1970).

323 Another important feature of the male pre-copulatory behavior is its relative consistency, as suggested
324 by Loher (1959) for the courtship behavior of male grasshopper in all Catantopinae species. This
325 stereotypical nature is evident in the present work from the high values of both the PO and TP
326 quantitative measurements. Orientation was followed by a slow, stealthy approach and a sudden jump in
327 the male's attempt to mount the female. Upon mounting, the male then displayed various stridulation
328 and vibration behavioral elements, culminating in copulation attempts and copulation.

329 Overall, male sexual behavior varied more during the pre-mounting than during the mounting sub-
330 stages. This was expressed in both, the larger repertoire of elements and the higher variability of their
331 occurrence (PO). A major behavioral element during pre-mounting was that of the slow elevation of the
332 hind legs (described previously in males of *Aulocara elliotii*; Bromenshenk & Anderson 1981). We
333 suggest that this element reflects the internal state of the male, i.e. sexual arousal and readiness to
334 mate (prior to mounting attempts). Limited courtship during pre-mounting was previously attributed to
335 both *S. gregaria* (Popov 1958; Strong & Amerasinghe 1977) and *Locusta migratoria* (Oberlin 1973 cited
336 in Strong & Amerasinghe 1977). Oberlin (1973) suggested that this is a result of the high inter-male
337 competition found under the crowded conditions of a locust swarm.

338 Stridulation (short- previously referred to as "short burst", "sharp sounds" or "assault-sounds"; and long-
339 previously referred to as "long sounds", "long burst", or "whizzing noises"; Loher 1959; Uvarov 1977)
340 and the hind leg (silent) vibration elements (referred to as "cycling of the hind legs"; Strong &
341 Amerasinghe 1977) are known as major characteristics of male sexual pre-copulatory behavior and
342 have been reported to feature during both pre-mounting and mounting (see also Norris 1954; Laub-
343 Drost cited in Uvarov 1977; Otte 1970). While their role is still not fully resolved, in our current
344 observations they were more frequent during mounting (as also mentioned by Loher, 1959). Overall, in
345 addition to its relatively shorter duration, the mounting stage seems to be the more conserved stage in
346 the locust's reproductive behavior.

347 Another intriguing behavioral element during pre-mounting is that of wing-fluttering. This was previously
348 reported for both sexes of the desert locust during sexual interaction ("stationary wings-fluttering" in
349 Loher 1959, Uvarov 1966, 1977; Njagi & Torto 2002). In other acridids wing fluttering was suggested to
350 have a role in mediating release of male volatile substances in relation to mate finding (Uvarov 1966).
351 However, the role of wing fluttering in relation to sexual behavior in the desert locust has not yet been
352 resolved.

353 *Female sexual behavior*

354 Female desert locusts demonstrated no clear courtship behavior, and were less dominant than males
355 during the sexual interaction (also reported by Norris 1964; Strong & Amerasinghe 1977; Inayatullah et
356 al. 1994). The sexual behavior of the females was also less stereotypic. Upon encountering a male,
357 female behavior comprised palp vibration, antennal movement, searching, and self-grooming. A central
358 characteristic of the female's behavior during both of the pre-copulatory stages was that of hind leg
359 vibration (Loher 1959; Strong & Amerasinghe 1977; Uvarov 1977). During pre-mounting, leg vibration
360 was mostly low amplitude, with less frequent intermittent high amplitude vibration. This is in
361 accordance with Loher's contention (1959) that the amplitude of this element reflects the level of
362 excitement of the locust (although, the role of this behavioral element in both sexes is still uncertain). It

363 was also suggested that the female's vibration of her hind legs may serve as a defensive response
364 against the male's mounting attempts. This is in accord with our major finding, suggesting that the most
365 prominent behavioral elements demonstrated by the females were those related to rejection of the
366 males.

367 *Sexual conflict*

368 In this study we paid particular attention to the behavior of females and males at the points of possible
369 conflict, preceding mate selection/decision. We suggest two points at which the conflict between the
370 sexes is manifested (traffic lights in Fig 3): the first occurs during pre-mounting and the second during
371 the mounting stage. The first point of conflict may actually appear repeatedly before a male's attempts
372 to mount the female, and is manifested in two elements: (1) the female's walking away ("running away"
373 in Loher 1959), and (2) jumping away (Popov 1958; also referred to as "leaping away" in Strong and
374 Amerasinghe, 1977). Jumping away better expresses rejection as it frequently followed dislodgment of
375 the male. We did not include kicking during pre-mounting, although intuitively it may serve as a primary
376 rejection element, because kicking is a common reflexive response of locusts, of both sexes, to tactile
377 stimuli by other locusts, regardless of sex (Norris 1962; Siegler & Burrows 1986).

378 When attempting to mount the female, males displayed two behavioral elements: (1) climbing
379 (described in this work for the first time), or (2) jumping (the more dominant behavior, previously
380 referred to as "attempt to copulate", "sexual attack", "copulation attack" or "assault"; Uvarov 1928;
381 Husain & Mathur 1946 cited in Popov 1958; Loher 1959; Pener 1967; Otte 1970). These two elements
382 were often preceded by peering or scanning (lateral swaying of the body from side to side). In both
383 larvae and adult locusts this behavior is related to estimating distance (Kennedy 1945; Wallace 1959).
384 Though not necessarily related to sexual interactions, scanning plays an important role in the pre-
385 mounting stage, serving the males when jumping, and also in the females' rejection response to an
386 approaching male.

387 Although, as mentioned above, the display of short stridulation was not very frequent during pre-
388 mounting, its appearance was commonly associated with dislodgement of the male by the mounted
389 female (in agreement with Loher 1959). Based on their differential relative appearance during pre-
390 mounting and mounting, our findings suggest different functional roles for the short and the long
391 stridulation. The overall role of auditory signaling in the courtship behavior of the male desert locust,
392 although previously considered as relatively insignificant (Loher 1959; Keuper et al. 1985; Robinson and
393 Hall 2002) would thus appear to be worth revisiting.

394 In the second point of conflict, during the mounting stage, the interactions between the sexes were
395 more complex. The females used both, direct and indirect rejection elements. Direct rejection
396 comprised jumping and kicking (defensive reaction, Loher 1959), commonly performed immediately
397 after the male had mounted the female, and often presented sequentially, promoting dislodgement of
398 the male from the female's back (repulsing the male, Loher 1959). In response to the female's kicking
399 behavior, a few males displayed defensive behavioral elements, including avoidance and blocking.
400 These latter two elements, described here for the first time, may have a major role in assisting the male
401 to overcome female rejection.

402 The indirect rejection by the female (passive phase, Strong & Amerasinghe 1977), comprising her
403 abdominal bending and abdominal grounding (the latter described here for the first time), is of special
404 interest as it drew a distinctive response from the male: i.e. pressing his hind legs firmly to the ground in
405 an attempt to lift the female.

406 We examined the efficacy of female jumping and kicking in successfully rejecting males at this conflict
407 point by preventing the females from using their hind legs. Constraining the females indeed resulted in
408 fewer mounting attempts and increased male mounting success. Hence we can safely postulate that a
409 major component of mate-choice by the female is based on consistent and vigorous rejection by way of

410 jumping and kicking. Males, however, overcome female rejection mostly by repeated mounting
411 attempts.

412 Throughout this study we did not detect any clear signal of female receptivity. High receptivity was best
413 demonstrated passively, whereby passive females did not reject the male (Popov 1958). Twisting of the
414 abdomen, suggested by Ballard et al. (1932; cited in Popov 1958) as a display of receptivity, was never
415 observed in the current study. Another issue that has remained unresolved is that of inter-sexual
416 recognition prior to pre-copulatory behavior. Previous reports have suggested mainly visual, but also
417 chemical, signaling as playing a role in mutual recognition between the sexes in the desert locust
418 (Popov 1958; Uvarov 1977; Pener & Shalom 1987; Obeng-Ofori et al. 1993; Franck & Schmidt 1994;
419 Inayatullah et al. 1994; Ely et al. 2006). Our findings support a major role of visual signals, as we
420 observed that rapid movement by the females (fast walking or jumping) appeared to enhance the males
421 sexual stimulation.

422

423 *Concluding remarks*

424 A detailed investigation of the sexual and reproductive behavior is a prerequisite for understanding the
425 evolutionary and ecological dynamics of a species (Kirkendall 1983; Thornhill and Alcock 1983). The
426 comprehensive description presented here of the reciprocal interactions between the sexes in the
427 desert locust thus contributes to our understanding of the biology and behavior of this economically
428 significant pest. The described and presented ethogram offers a tool with which to compare behavioral
429 similarities and differences among different orthopteran insects (Paranjape 1985), and specifically
430 among locust species. Here we exclusively described the sexual behavior of the desert locust in the
431 gregarious phase. The knowledge acquired in this study and the tools developed for it will be used for a
432 future comparative investigation of locusts in the gregarious and solitary phases, emphasizing the
433 different features of the sexual conflict in relation to the phase phenomenon.

434 As noted, the desert locust is one of the most notorious agricultural pests. Major efforts have been
435 invested in investigating the sexual behavior of pest insects (Walgenbach & Burkholder 1987; Rojas et
436 al. 1990; Zahn et al. 2008), with the rationale being that a better understanding of their sexual and
437 reproductive behavior will contribute to the application of pest management (Boake et al. 1996;
438 Suckling 2000). This work may thus also assist in identifying novel targets and generating
439 environmentally friendly methods for locust control.

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446

447 **Author Contribution:**

448 YG performed the experiments, prepared figures and tables. YR prepared figures and tables. AH AA and YG
449 conceived and designed the experiments. All authors wrote and reviewed drafts of the paper

450

451 **Disclosure:**

452 The authors state that they have no conflicts of interest, including specific financial interests, relationships or
453 other affiliations, relevant to the reported research and results.

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Table 1. The behavioral repertoire during the pre-mounting stage.

Behav. Element	Description	Sex		Citation
		m	f	
Grooming	Eyes, antennas, mouth part, mid and front legs, wings and abdominal grooming	+	+	O'Shea 1970; Rowell 1971; Uvarov 1977; Berkowitz & Laurent 1996
Antennal movement	Movement of the antennas	+	+	Loher 1959; Wallace 1959
Palp vibration	Vibration movements of both labial and maxillary palps	+	+	Uvarov 1977
Searching	Combination of walking, scanning, antennal movement and palps vibration	+	+	based on Inayatullah et al. 1996
Orienting	Anterior side is directed towards the female	+	-	Otte 1970; Amerasinghe 1978
High hind leg (HL) femoral vibration	Femur is lifted to a perpendicularly position relatively to the ground (~90°) while vibrating the hind legs.	+	+	Loher 1959
Low hind leg (HL) femoral vibration	Femur is lifted to a perpendicularly position relatively to the ground (<90°) while vibrating the hind legs.	+	+	Loher 1959
Wing flapping	Flapping movements of both wing pairs	+	+	Wies-Fogh 1956a,b cited in Uvarov 1966; Uvarov 1977
Long stridulation	Rapid vibration of the wing-pairs producing whizzing noise	+	-	Loher 1959
Short stridulation	Wings beating against	+	-	Loher 1959
Abdomen wagging	Wagging (mostly lateral) movements of the abdomen	+	+	*
Genital opening	Rhythmic opening of the genital-opening	+	+	*
Initiating physical contact	Locusts touching each other	+	+	Popov 1958
<i>Mutual antennal contact</i>	Locust antennas touching each other	+	+	*
Slow repeating hind leg elevation	Slow elevation of the hind legs	+	-	*
Approaching	Walking clearly directed towards other sex	+	+	Popov 1958; Loher 1959
Walking away from the male	Distancing from the male by walking	-	+	Popov 1958; Loher 1959
Jumping away from the male	Distancing from the male by jumping	-	+	Popov 1958; Loher 1959
Mounting by Climbing	Attempting to mount the female by climbing	+	-	*
Mounting attempt by Jumping	Attempting to mount the female by jumping	+	-	Uvarov 1928; Husain & Mathur 1946

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Behaviors which are shared and mutually exhibited by both sexes are presented in italic bold font. * Represents elements which are described for the first time.

461 **Table 2. The behavioral repertoire during the mounting stage.**

Behav. Element	Description	Sex		Citation
		m	f	
<i>Mounting</i>	The male mounting the female/ female is being mounted by the male	+	+	Uvarov 1928; Husain & Mathur 1946
Avoidance	Elevation of the two hind legs femur close together in the air	+	-	*
Blocking	Lifting both Hind legs in the air perpendicularly to the ground, femur and tibia folded	+	-	*
Jumping	Jumping while carrying the male	-	+	Loher 1959
Kicking the male	Directed kicking towards the male	-	+	Loher 1959
Abdominal bending	Abdominal tips and genital are bended sideways	-	+	Strong & Amerasinghe 1977
Abdomen grounding	Abdomen is pressed to the ground	-	+	*
<i>Male's dislodgement</i>	Male is dislodged from the female's back	+	+	Popov 1958; Uvarov 1977
Lifting attempt	Males attempt to lift the female by pushing against the ground and straightening of the hind legs	+	-	*
Antennal movement	Movement of the antennas	+	+	Uvarov 1977
Palp vibration	Vibration of both labial and maxillary palps	+	+	Loher 1959
Hind legs vibration	Femur is being positioned perpendicularly to the ground while tibia is flexed 90° to the femur.	+	-	Loher 1959
Short Stridulation	Wings beating against each other resulting in short and sharp sounds	+	-	Loher 1959
Long stridulation	Rapid vibration of the wings while in resting position	+	-	Loher 1959
Lateral hind leg vibration	Lateral vibration of the hind legs femur	-	+	Loher 1959
Hind leg grounding	Pushing the hind legs against the ground.	+	-	*
Genital opening	Rhythmic opening-closing of the genital opening	+	+	*
Copulation attempt	Male's abdomen is placed to the side of the female's ones in order to reach her genitalia	+	-	Loher 1959
<i>Copulation</i>	Copulation defined as the time when sperm is transferred			Pener 1967

462 Behaviors which are shared and mutually exhibited by both sexes are presented in italic bold
 463 font. * Represents elements which are described for the first time.

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797 **Figures Captions:**

798

799 **Figure 1**

800 The precopulatory behavioral repertoire of the male (left) and female (right) desert locust. The pre-
801 mounting and mounting behavioral elements are listed from step 1 to 7 (S1-S7) and color coded
802 according to relevant body part. The probability of an element occurring (PO %; mean + SEM) is shown.
803 Behavioral elements that are shared and mutually exhibited by both sexes are presented in italic bold
804 font.

805

806 **Figure 2**

807 Representative behavioral elements observed during the pre-mounting (A-B) and mounting (C-E)
808 stages: A. Male slow repeated hind leg elevation. B. Female hind leg low and high-amplitude vibration.
809 C. Male short and long wing stridulation. D. Male hind leg vibration and copulation attempt. E.
810 Successful copulation. The animations in A-D were drawn from images taken from video sequences.

811

812 **Figure 3**

813 An ethogram depicting the desert locusts pre-copulatory interactions leading to copulation. The male
814 behavior is on the left, and that of the female on the right. S1-S8 indicate the chronological step number
815 during the pre-mounting and mounting stages. Traffic lights denote points at which female choice takes
816 place (steps 3 and 6); Red is associated with rejection of the male. Green is associated with the female
817 tolerating the male.

818

819 **Figure 4**

820 Examples of female rejection behaviors and male responses during the second point of mate choice
821 (second traffic light in the ethogram in Fig. 3). A. Male attempts to block the female's kicking using his

822 hind legs. B. Female displaying lateral abdomen bending behavior while also kicking, and male
823 responding to kicking by avoidance behavior. C. Female pressing her abdomen to the ground to avoid
824 mating (i.e. 'abdominal grounding'). D. Male managing to mate with the female by pushing with his hind
825 legs and lifting her.

826

827 **Figure 5**

828 A kinematic diagram depicting the pre-copulatory behavior of male locusts (N=20); arrows represent
829 transitions between behavioral elements. The numbers on a gray background denote the mean
830 transitional probability (TP) between each pair of behavioral elements. Two way transitions are depicted
831 by double-headed arrows (numbers relate to the closer arrow head). The color of the circles
832 representing the different behavioral elements corresponds to the color index used in Figure 1. The
833 different steps in the pre-mounting and mounting stages are noted.

834

835 **Figure 6**

836 A kinematic diagram depicting the sexual behavior of female locusts (N=20); details as in figure 5.

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