



Olfactory Mating Signals in the Migratory Locust *Locusta migratoria*

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Abstract

Swarming locusts cause huge plagues across the world threatening food production. Before swarms form, locust populations exhibit a dramatic phase change from a solitary to a gregarious phase. The cause of this phase change is a complicated interplay of conspecific and environmental cues and is, especially for one of the major pests, the migratory locust *Locusta migratoria*, still not well understood. Here we study the behavior of both solitary and gregarious *L. migratoria* towards the headspace odors of conspecifics. As we do not find a general attraction of gregarious animals to the headspace of gregarious conspecifics, swarm formation does not seem to be mainly governed by olfactory aggregation cues. When testing for potential mating signals, we observe that the headspace of virgin gregarious females is highly attractive only towards virgin males of the same phase, while mated gregarious males and solitary males, regardless of their mating state, do not become attracted. Interestingly, this phase-specific attraction goes along with the finding, that mating behavior in experiments with inter-phasic pairings is extremely rare. Our data suggest that odor emissions in *L. migratoria* play a significant role in a mating context.

Keywords Migratory locusts · Chemical ecology · Mating · Aggregation · Behavior

Introduction

The devastating effect of swarms of gregarious locusts has been reported since biblical times. While solitary and gregarious locusts had been considered to be different species, in 1921, Uvarov discovered the fascinating phenomenon of phase polyphenism (Pflüger and Bräunig 2021; Simpson et al. 2011). As we now know from several locust species (Topaz et al. 2012), *Locusta migratoria* (Linnaeus 1758; Order: Orthoptera, Family: Acrididae) exists in solitary and gregarious phases. These phases differ in morphological, anatomical, and behavioral features (Greenwood and Chapman 1984; Latchininsky 2019; Wang et al. 2014; Wei et al. 2017), with e.g., the solitary phase being more camouflaged, while the gregarious phase is conspicuously colored. The shift between both phases is a complex

phenomenon, that to some extent is still considered a puzzle. In desert locusts *Schistocerca gregaria* (Forsskål 1775; Order: Orthoptera, Family: Acrididae), factors such as environmental cues and sensory cues from conspecifics, including visual, tactile, and olfactory cues, seem to be involved (Nakano et al. 2022; Roessingh et al. 1998; Simpson et al. 2011). The role of olfaction in the locusts' phase polyphenism has been explored over the past few decades (Guo et al. 2020; Wei et al. 2017). It is well understood that odor profiles are dynamic within the life stages and between the phases (Wei et al. 2017). Most of the existing literature, however, focuses on *S. gregaria* and suggests that headspace odors from gregarious animals are attractive to gregarious and repulsive to solitary conspecifics (Roessingh et al. 1993, 1998; Rogers et al. 2003). However, as *S. gregaria* and *L. migratoria* even differ in their responses to the pivotal body odor phenylacetoneitrile (PAN) that is present in both species (Pener and Simpson 2009; Torto et al. 1996; Wei et al. 2019), the general function of headspace odors in *L. migratoria* so far remains elusive. Moreover, phase shift dynamics also differ directionally between the phases in *S. gregaria* (Simpson et al. 1999). However, there is a lack of understanding about *L. migratoria* that prompts further research to understand the behavioral response of *L. migratoria* to conspecific smells.

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57 Here, we provide headspace odor collections of differ- 84
 58 ent developmental stages of gregarious *L. migratoria* to 85
 59 individual animals in a binary choice arena. Some studies 86
 60 have so far focused on the behavioral responses towards 87
 61 animal-released odors mainly in *S. gregaria* (Obeng-Ofori 88
 62 et al. 1993; Torto et al. 1996). We, therefore, performed a 89
 63 comprehensive set of experiments to test, whether stage-,
 64 phase-, and/or sex-specific odor blends provoke attraction
 65 or repulsion to the different stages, phases, and sexes in *L.*
 66 *migratoria*. At the same time, interphasic mating occurs in
 67 *S. gregaria* (Golov et al. 2018a) but this phenomenon has
 68 not been studied in *L. migratoria*. We, therefore, extend our
 69 study to mating assays between the different phases of *L.*
 70 *migratoria*. In conclusion, we aim to increase our under-
 71 standing of *L. migratoria* with regard to the behavioral
 72 impact of its body odors.

aging, to finally reach around 200 adult animals in the same
 cage. The solitary animals were separated on the day of
 hatching into individual cylindrical boxes (height, 10.5 cm;
 diameter 8 cm). Each solitary animal was supplied with
 a separate ventilation system. Both phases were fed with
 wheat grass provided by our greenhouse.

Bioassay Arena

The behavioral setup includes a cuboid base, an arena sur-
 face consisting of a mesh, and an arena enclosure (Fig. 1a).
 The base of the behavioral setup consists of two separate
 polypropylene boxes (A) (16 cm × 30.5 cm × 25 cm) with
 air diffusers opening upwards at the middle of the lower
 surface (B) of each of the boxes. The diffuser is connected
 to the odor/control source (C). The base is high enough to
 evenly distribute the odor at the base before the air enters the
 arena. The in-house air is controlled by two flowmeters con-
 nected to the odor and control source via a 6/4 mm Teflon
 pipe. The air inlet is kept at 3L/min for each side. From the
 source, the air is introduced to the base of each side of the
 setup through the diffuser. Two perforated polypropylene
 plates of size 25 cm × 30 cm, with perforations of 2 mm
 diameter and distributed evenly at every 2 mm are placed on
 each of the two boxes making a rectangular behavioral arena
 (D). The arena has no division in the middle, allowing the
 locust to move in all directions within the arena. This design
 results in an arena divided into two zones, one with odor
 and one without that the animal can chose depending on the
 valence of the tested odor. The arena is enclosed by another
 rectangular polypropylene box (26 cm × 62 cm × 39 cm)

73 Methods and Materials

74 Animal Breeding

75 We used *L. migratoria* that we bought from a local pet shop.
 76 The gregarious and solitary animals used for tests were kept
 77 separated for a minimum of 5 generations. Both phases were
 78 maintained at the Max Planck Institute for Chemical Ecology
 79 in climate chambers with a 14:10 h light:dark cycle, at a
 80 temperature of 30 ± 2 °C, and humidity of $50 \pm 5\%$. The gre-
 81 garious animals were kept with around 300–400 first instar
 82 animals in a cubic cage (side length, 30 cm × 30 cm × 30 cm).
 83 The numbers of animals were reduced continuously during

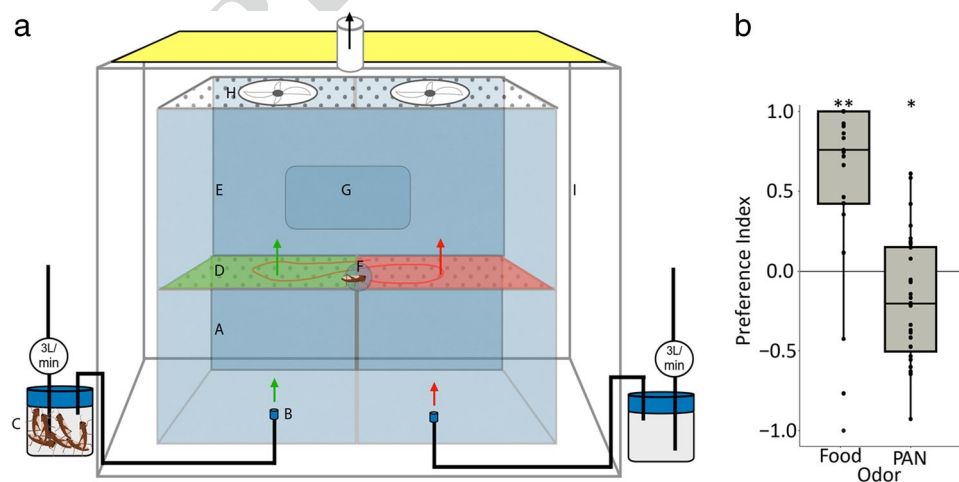


Fig. 1 Assay for testing attraction and aversion of odors. **a** Experimental setup: A, polypropylene base; B, air diffusers; C, odor source; D, odor (green) and control (red) zones of arena; E, arena enclosure; F, opening to introduce animal; G, window to remove animal; H, axial fan; I, enclosing chamber. **b** Gregarious *L. migratoria* nymphs show significant attraction to food odors ($n=23$, $p=0.0018$,

Wilcoxon-signed rank test) and repulsion to PAN ($n=31$, $p=0.017$, *Wilcoxon-signed rank test*). Box represents the 50% of the central data/interquartile range (IQR) with median, the whiskers represent the range (= upper quartile+1.5 IQR/ lower quartile-1.5IQR). ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$

113 to limit the locust within the arena (E). A circular inlet of
 114 5 cm diameter that can be opened and closed from outside
 115 is situated at the same level as the behavioral arena plane
 116 to introduce locusts into the arena (F). A rectangular open-
 117 ing of 29 cm × 25 cm with a closing door is situated above
 118 the animal inlet to retrieve the animal after each trial, with
 119 the least disturbance to the airflow (G). The base, arena,
 120 and enclosing walls are supported with aluminum hinges
 121 of 1 cm width. A pair of axial fans (H) (connected to the
 122 same voltage input) are suspended on top of each side 39 cm
 123 above the arena to ensure a laminar and vertical airflow (ca.
 124 3 cm/s) in the arena. Between the fans, an HD USB camera
 125 covering the whole arena is fitted and connected to a com-
 126 puter outside. The whole setup is placed inside an enclosing
 127 chamber 92*72*65 cm with solid white walls to exclude
 128 visual distraction from outside of the arena (I). LED light
 129 is fitted 11 cm above the enclosure to ensure even lighting
 130 throughout the arena surface. The air from the enclosure is
 131 ventilated out at a constant rate of approximately 30L/min
 132 via an exhaust fitted 15 cm above the behavioral setup. The
 133 setup is maintained at $31 \pm 1^\circ\text{C}$ and humidity at 50% during
 134 the trials.

135 Bioassay Procedure

136 For each trial, one animal was introduced into the arena
 137 through the animal inlet, and each animal was tested only
 138 once. If an animal refused to enter the arena within the first
 139 5 min, it was removed, and a new trial was started with a
 140 new animal. The 10-min recording of the animal's behavior
 141 started only after the animal made an initial movement of
 142 at least 1 cm or more into the arena. After every 5 trials, the
 143 arena was wiped with 70% ethanol and ventilated for an hour
 144 with clean air to remove any odors and any potential trails
 145 left by the animals.

146 20 unstarved animals in an air-tight box were used as an
 147 odor source. After every 5 trials, the animals used as the
 148 odor source were placed back into the breeding cage for an
 149 hour after which they were again used as the odor source in
 150 the setup. In all tests, the side of odor within the arena was
 151 reversed after half of the trials. The nymphs used were all in
 152 the late fourth instar and not separated by males or females.
 153 The virgin animals for experiments were taken 6–8 days post
 154 eclosion and the mated animals were taken a few hours after
 155 the male debarks the female or the female finishes oviposi-
 156 tion. The solitary animals were simply marked on the cage
 157 on the date of eclosion and used 8 days later for the tests.

158 Mating Experiments

159 To measure the willingness to mate, we used mounting
 160 behavior by males (virgin animals 6–8 days after the final
 161 eclosion) as a parameter. The animals were kept together

in the cage and were observed until the first mounting hap- 162
 163 pened. The mounted pair was removed and the remaining
 164 animals were separated into males and females and used for
 165 experiments within 48 h of separation. Mating tests were
 166 done in a 10 cm × 10 cm cage built from perforated plates
 167 on 5 sides and a glass on one side allowing observation.
 168 Each cage, considered as one data point, consists of four
 169 males and five females. The proportion of the number of
 170 males mounting the females was observed at 6 and 12 h.
 171 This was done inter-phase as a test and intra-phase as control
 172 experiments.

Data Analysis 173

In the odor choice bioassay procedure, the time (in seconds) 174
 175 an individual animal spent in the control or the odor side
 176 was observed. We considered the exact middle of the setup
 177 to be the division and the place of the head of the animal
 178 as the animal's location when the animal spent time in the
 179 2 cm-wide fringe area of the two zones.

The preference index was calculated as: 180
 $PI = (Time_{inOdor(s)} - Time_{inControl(s)}) / (Totaltime(600s))$. To test whether the 181
 182 preference was significantly attractive or aversive, we used
 183 the *Wilcoxon-signed rank test*.

Mounting behavior was analyzed by the *Mann Whitney-U* 184
 185 *test*, at 6 h. To compare between multiple groups, at 12 h,
 186 *Kruskal–Wallis test with Dunn's posthoc test* for multiple
 187 comparisons was used. In all cases, both for *PI* and mat-
 188 ing experiments, ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$.
 189 *Wilcoxon-signed rank test* was performed in R version 4.2.3.
 190 For multiple group comparison GraphPad InStat was used.

PAN used for the experiments was ordered in the highest 191
 192 purity available from Sigma Aldrich (B19401-250G).

Results 193

Evaluation of Bioassay 194

To screen for the behavioral valence of body odors, we used 195
 196 a two-zone arena, where the headspace of 20 animals from
 197 a given stage was infused to one side, while the other side
 198 was infused with control air (Fig. 1a). The time spent by
 199 individual animals in either zone was measured to investi-
 200 gate whether the odor was perceived as attractive (i.e., more
 201 time spent on the odor side), or repellent (i.e., more time
 202 spent on the control side).

To test the assay for functionality, experiments were first 203
 204 performed with an attractive food odor (i.e., headspace emit-
 205 ted by 5 g of shredded wheat grass) and with the known
 206 repellent PAN (100 μL at 1 mg/mL concentration) diluted
 207 with mineral oil. PAN is repulsive in this assay to all stages
 208 and phase of the *L. migratoria* (Chang et al. 2023). When

209 testing starved fourth instar gregarious nymphs with the food
210 odor, the animals showed strong attraction to the odor and
211 spent significantly more time on the side of the arena smell-
212 ing of wheat (Fig. 1b). Animals of the same cohort tested
213 with PAN avoided the side with this odor (Fig. 1b), demon-
214 strating that the assay indeed was suitable for testing both
215 attraction and aversion.

216 Responses of Nymphs in Bioassay

217 Gregarious locusts usually aggregate in conspicuous huge
218 hopper bands or swarms, whereas locusts of the solitary
219 phase avoid groups and are rather cryptic. To investigate
220 whether the aggregation and repulsion in gregarious and
221 solitary animals, respectively, is governed by olfactory
222 cues from the gregarious nymphs, the valence of odors
223 from gregarious nymphs was tested in gregarious and soli-
224 tary nymphs. Surprisingly, we did not find any significant
225 response either in gregarious or in solitary nymphs to the
226 headspace of gregarious nymphs (Fig. 2a). Similarly, no
227 other tested headspaces from gregarious adults, except for
228 the headspace of gregarious virgin females, elicited any sig-
229 nificant attraction in gregarious or solitary nymphs (Supple-
230 mentary Fig. 1). It thus seems that olfactory cues alone are
231 not sufficient for the forming of hopper bands in gregarious
232 *L. migratoria*.

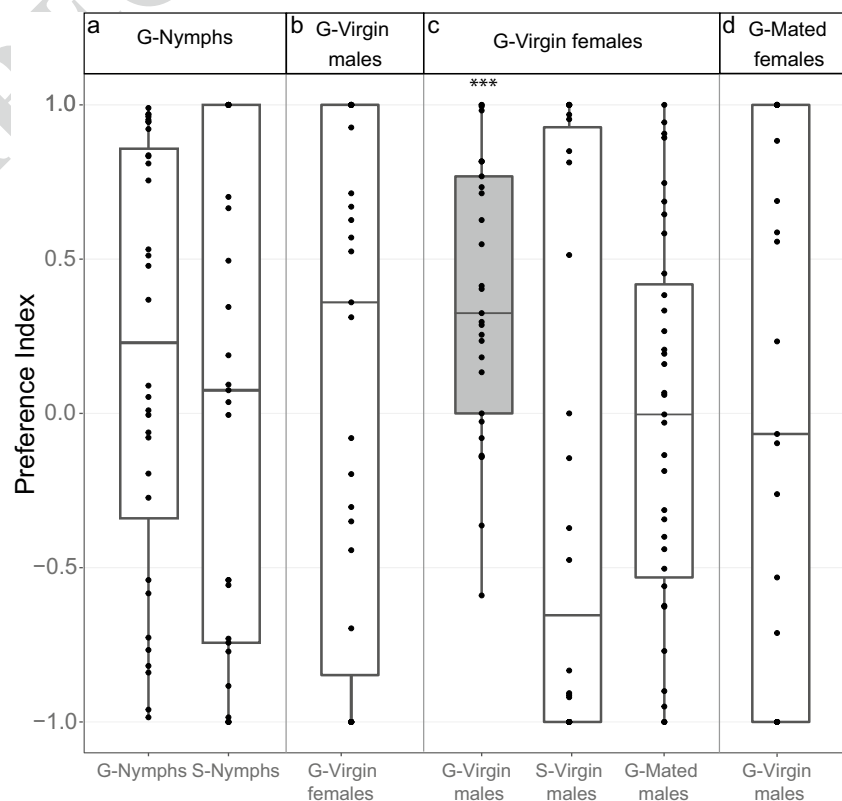
Responses of Adults in Bioassay

234 We next asked whether attraction towards potential mates
235 might be governed by olfactory cues. When testing gregari-
236 ous virgin males and virgin females with the headspace of
237 the opposite sex, males were significantly attracted to the
238 female odor, while females did not respond to the male odor
239 (Fig. 2b, c). Interestingly, when testing for inter-phase attrac-
240 tion, we found that the odors from gregarious virgin females
241 did not elicit any response in solitary virgin males (Fig. 2b).
242 In addition, contrary to the headspace of virgin gregarious
243 females, the odor emitted by mated gregarious females was
244 not attractive to virgin gregarious males (Fig. 2d), suggest-
245 ing that the headspace of mated females is either lacking
246 attractive compounds or includes repellent compounds that
247 virgin females are not emitting. At the same time, the attrac-
248 tion of gregarious males towards the odor of virgin gregari-
249 ous females was diminished when the males were already
250 mated (Fig. 2c).

Mating Experiments

251 Having found that solitary males showed no attraction to
252 the headspace of virgin gregarious females, we tested if this
253 results in an inter-phase mating barrier. We conducted mat-
254 ing experiments, where virgin males were paired with either
255 virgin females of the same or opposite phase (Fig. 3a). We
256

Fig. 2 Attraction of animal headspaces in *L. migratoria*. **a** Preference of gregarious ($n=32$, $p=0.13$) and solitary nymphs ($n=29$, $p=0.66$) tested with headspace odors of gregarious nymphs. **b** Gregarious virgin females ($n=31$, $p=0.50$) tested with odors of gregarious virgin males. **c** Gregarious virgin males ($n=29$, $p=0.0004$), solitary virgin males ($n=30$, $p=0.25$), and gregarious mated males ($n=35$, $p=0.74$) tested with the odor of gregarious virgin females. **d** Gregarious virgin males ($n=27$, $p=0.95$) tested with odors of gregarious mated females. Wilcoxon-signed rank test was used to determine P values. Box represents the 50% of the central data/interquartile range (IQR) with median, the whiskers represent the range (= upper quartile + 1.5 IQR/ lower quartile - 1.5IQR). ***, $p < 0.001$



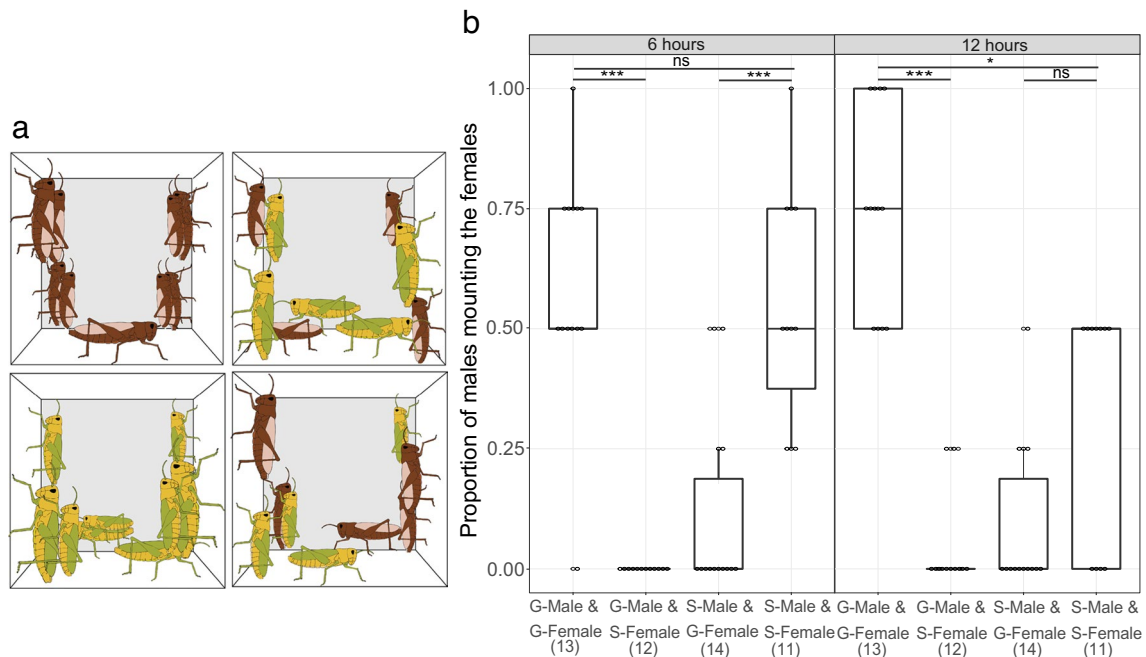


Fig. 3 Intra- and inter-phase mating behavior in *L. migratoria*. **a** Schematic presentation of the mating experiment for an overview of the crowding of animals in the experiment. Cubic cage (side lengths 10 cm), experimental animals drawn to scale. The gregarious animals are represented in brown while the solitary animals are green and yellow. **b** Proportion of males mounting females in the intra- and inter-

phase combinations of solitary and gregarious animals observed after 6 (left) and 12 (right) hours. *Kruskal–Wallis test with Dunn’s posthoc test* for selected comparisons. Box represents the 50% of the central data/interquartile range (IQR) with median, the whiskers represent the range (= upper quartile + 1.5 IQR/ lower quartile - 1.5 IQR). ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$

257 then observed the mounting behavior which is an obliga- 279
 258 tory and easily observable step in orthopteran mating. 280
 259 Interestingly, we found that the frequency of mounting was 281
 260 significantly lower in inter-phase tests. At the end of 6 h, 282
 261 while 59% of the gregarious males mounted gregarious 283
 262 females, only 10% of the solitary males mounted the gre- 284
 263 garious females. At the same time, 54% of the solitary males 285
 264 mounted the solitary females, while none of the gregarious 286
 265 males mounted solitary females during the 6 h of observa- 287
 266 tions (Fig. 3b). While intra-phase combinations resulted in 288
 267 similar mounting ratios in gregarious and solitary animals 289
 268 during the first 6 h of the experiments, the mounting behav- 290
 269 ior of gregarious animals lasted longer, as many solitary 291
 270 males had already unmounted their solitary females by the 292
 271 end of 12 h (Fig. 3b). 293

272 Discussion

273 The phase state in locusts has been shown to be governed 294
 274 both by environmental cues and cues from conspecifics 295
 275 (Nakano et al. 2022). From a sensory point of view, differ- 296
 276 ent developmental stages of *S. gregaria* aggregate based on 297
 277 both chemo- and mechanosensory cues (Niassy et al. 1999; 298
 278 Rogers et al. 2003; Simpson et al. 2001). Contact cues and 299
 300
 301
 302
 303

short-range odors seem to be pivotal in the gregarious phase, 279
 while long range auditory cues are more important in the 280
 solitary phase (Nakano et al. 2022; Pener and Simpson 281
 2009). The short-range odors emitted and perceived by the 282
 gregarious animals emanate from food, feces, and the head- 283
 space of conspecifics. Based on these results we expected, 284
 that gregarious nymphs of *L. migratoria*, which form hopper 285
 bands, are attracted by the headspace of gregarious nymphs. 286
 Surprisingly, we did not find any attraction, and gregarious 287
 nymphs in most aspects did not differ from solitary ones, 288
 which were neither attracted nor repulsed by any of the gre- 289
 garious headspaces. 290

S. gregaria have been reported to shift easily from the soli- 291
 tary to the gregarious phase (Rogers et al. 2003), whereas 292
 for *L. migratoria*, the solitary phase seems to be the more 293
 stable state, and a shift to the gregarious phase is difficult 294
 to induce and needs an extremely high density of animals. Cor- 295
 responding to that the rate of gregarization and solitarisation 296
 is also different within and between locust species (Topaz 297
 et al. 2012). *S. gregaria* exhibit a much faster gregarisation 298
 and a slower solitarisation process (Simpson et al 1999; Wei 299
 et al. 2017). A higher concentration of odors or even a com- 300
 bination of multi-modal cues including e.g., mechanosen- 301
 sory or visual signals might thus potentially be necessary to 302
 trigger aggregation behavior among *L. migratoria* nymphs. 303

304 The headspace composition has been shown to be
 305 dynamic and dependent on phase and state (Wei et al.
 306 2017). 4-vinylanisole (4 VA) is one of the headspace odors
 307 that has been identified as a possible aggregation phero-
 308 mone in *L. migratoria* (Guo et al. 2020). PAN is another
 309 dominant headspace odor in both *L. migratoria* and *S.*
 310 *gregaria*. The role of PAN in *S. gregaria* is still under
 311 debate, as some studies suggest a role as an aggregation
 312 pheromone (Torto et al. 1994), others supposed repellency
 313 (Seidelmann et al. 2005), while yet others even suggest a
 314 role in sexual behavior (Seidelmann and Ferenz 2002).
 315 In *L. migratoria*, however, PAN is repulsive to animals
 316 of all stages and phases (Chang et al. 2023; Wei et al.
 317 2019). The responses of nymphs that we observed in the
 318 aggregation tests could thus be expected, considering the
 319 odor profile of individual groups. The higher proportion of
 320 PAN to 4VA in gregarious males as compared to gregari-
 321 ous females (Wei et al 2019) could form a background to
 322 the neutral response to gregarious male odors, where the
 323 attractant 4VA and the repellent PAN balance each other.
 324 The strongly attractive response by gregarious nymphs to
 325 gregarious females could rely on the higher amount of
 326 4VA, which would dominate over the repellency of PAN.
 327 Similar mixture interactions in binary mixtures of oppos-
 328 ing valence have also been reported in e.g., *Drosophila*
 329 (Mohamed et al. 2019; Thoma et al. 2014).

330 When looking for potential effects of headspace odors on
 331 mating behavior, we found a strong attraction of gregarious
 332 males to the headspace of virgin gregarious females. As the
 333 number of gregarious males is much higher than that of greg-
 334 arious females in mating swarms and in ovipositing popula-
 335 tions (Ellis and Ashall 1957), gregarious males face strong
 336 competition for females. The indifferent response of the vir-
 337 gin gregarious females to the headspace of virgin gregari-
 338 ous males is coherent with this proportion, as females do not
 339 have to actively respond to males for successful mating. The
 340 lack of response of virgin gregarious males to mated females
 341 could indicate the presence of a courtship inhibitory factor.
 342 Such a factor can be either produced by the female herself to
 343 prevent further harassment from males (Engel et al. 2016)
 344 or be transferred by the male during mating as an additional
 345 passive form of mate guarding (Seidelmann 2006). Male-
 346 transferred anti-aphrodisiacs are found in other insects too
 347 (Khallaf et al. 2020, vander Meer et al. 1986). In *S. gregaria*,
 348 PAN acts as such a courtship inhibition pheromone (Seidel-
 349 mann and Ferenz 2002). Alternatively, mated females could
 350 emit lower amounts of pheromones. The lack of response
 351 by mated gregarious males to the odor of virgin gregarious
 352 females could be interpreted as a sign of transient abstinence
 353 during a recovery phase (Barrozo et al. 2010a) which has been
 354 shown in other insects to be combined with a lower sensitivity
 355 to female pheromones post mating (Barrozo et al. 2010b).

356 When testing mating behavior within the phases, we already
 357 found differences between the gregarious and the solitary ani-
 358 mals. The mounting of solitary males usually lasted for less
 359 than 12 h, while that of gregarious males lasted much longer.
 360 Mounting of females by males is the longest copulation step and
 361 is present in both phases. It has been proposed as an active mate
 362 guarding strategy of the male to avoid remating of the female
 363 before his sperm has fertilized her eggs (Golov et al. 2018a;
 364 Zhu and Tanaka 2002). The duration of the mounting varies
 365 depending on sub species, duration of separation of males into
 366 cohorts, and phase (Golov et al. 2018a, b; Seidelmann 2006;
 367 Tanaka and Zhu 2003). The longer mounting that we observed
 368 in the gregarious phase could, hence, be interpreted as an effect
 369 of the higher male-male competition faced by gregarious males
 370 within the swarm.

371 In the inter-phase attraction experiments, we found it
 372 intriguing that solitary males were not attracted by the
 373 headspace of virgin gregarious females. We, therefore,
 374 asked whether males and females from different phases
 375 would mate at all. In *S. gregaria* gregarious males exhibit
 376 frequent mounting attempts when encountering solitary
 377 females. Interestingly, we found an opposite trend in *L.*
 378 *migratoria*. Males of a given phase showed only weak
 379 interest in females of the other.

380 In conclusion, we found that odors emitted by nymphs
 381 seem to be of less importance in attracting other nymphs
 382 into hopper bands. Other sensory cues or odors of a higher
 383 concentration, or combinations of these might be the
 384 deciding factors. The attraction between the sexes does,
 385 however, seem to be relying on female-produced cues as
 386 gregarious males were strongly attracted to the odor of vir-
 387 gin gregarious females. Interestingly, solitary males were
 388 not attracted to the smell of gregarious females, revealing
 389 a certain degree of an inter-phase mating barrier. This pos-
 390 tulation was further corroborated by our finding that male
 391 mounting behavior in couples of mixed phases is very rare.

392 **Supplementary Information** The online version contains supplement-
 393 ary material available at <https://doi.org/10.1007/s10886-023-01456-9>.

394 **Author's Contributions** All authors were involved in the design of the
 395 study; APU performed the experiments; APU and MK analyzed the
 396 data; APU and MK wrote the first draft of the manuscript; all authors
 397 contributed to the final version of the manuscript.

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400 **Data Availability** Data will be made available on request.

401 **Declarations**

402 **Ethical Approval** Not applicable.

403 **Competing Interests** The authors have no competing interests to
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 405 no relevant financial or non-financial interests to disclose.

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