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The courtship-inhibiting pheromone is ignored by female-deprived gregarious desert locust males

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Gregarious mature males of the desert locust (*Schistocerca gregaria*) emit a courtship-inhibiting pheromone continuously to repel rivals. This signal evokes a strong response from males with recent experience of mature females. However, if males have been female deprived for some time, they start to ignore the pheromone and attempt to usurp females that are guarded by males. The probability and intensity with which males struggle for an occupied mate was found to depend on the time previously spent without a female. This adaptive behaviour is similar to the response to host-marking pheromones in phytophagous insects and parasitoids.

Keywords: courtship-inhibiting pheromone; postcopulatory guarding; signal ignorance; mating strategy; desert locust

1. INTRODUCTION

In competitive situations, pheromones function to inform the recipient about the state of, or action by, the sender. The recipient decides according to its physiological state (influenced by either internal and/or external factors) whether and how to respond to the pheromone (Nufio & Papaj 2001; Hofmeister & Roitberg 2002). In functional terms, this decision involves weighing up the fitness costs and benefits of responding to or disregarding the signal.

Courtship-inhibiting pheromones (CIPs) are signals used in a competitive context. They are produced by the sex with the larger variance in reproductive success (usually males) to signal that a potential mate (usually a female) is occupied and will be defended by the signaller (active mate guarding). Noteworthy pheromones with a similar function are usually called an anti-aphrodisiac when males transfer the pheromones to females (passive mate guarding). In such systems, the female has been rendered non-responsive and will refuse further mating attempts (e.g. Happ 1969; Gilbert 1976; Andersson *et al.* 2003). The recipient has to decide whether to ignore the signal and try to mate nevertheless, or to pay attention to it and search for another mating opportunity. This decision involves costs and benefits: if the possibility of finding another mate and fathering offspring is higher than the possibility of taking over the occupied mate and succeeding in sperm competition with the displaced competitor, it pays to heed the signal. Otherwise, the signal should be ignored.

I tested this hypothesis in *Schistocerca gregaria* (Orthoptera, Acrididae), a locust showing density-dependent phase polyphenism (Uvarov 1977). Mature gregarious males produce and release phenylacetonitrile (PAN, syn. benzyl cyanide) as a CIP to chemically enhance their active postcopulatory contact mate guarding (Seidelmann & Ferenz 2002; Seidelmann *et al.* 2005). The pheromone is released continuously in response to the presence of other mature males, irrespective of any copulations or mating attempts. Isolated males (gregarious or solitarious) do not release PAN (Seidelmann *et al.* 2000). The pheromone was demonstrated to deter males with recent experience of potential mates from takeover attempts of male-guarded females (Seidelmann & Ferenz 2002). However, if males are female deprived by having been kept in male-only groups, they might be expected to ignore the CIP and attack a couple in order to takeover the receptive female.

2. MATERIAL AND METHODS

Experiments were done with gregarious *S. gregaria* locusts taken directly from a long-term crowd-reared culture (see Seidelmann *et al.* (2000) for origin and conditions of culture). I used only mature locusts at the age of 20–30 days after final moult that were engaged in pairing to ensure the right stage of development (females close to oviposition) and intensive sexual behaviour (males willing to mate and to attack couples not protected by PAN; see Seidelmann & Ferenz 2002). A couple was placed in an open arena (660 cm^2). After pair reformation (many couples separated owing to handling), a test male was introduced. Test males were from the same culture and age (age span max. 4 days). They had been deprived of females in groups of 10 in small cages ($20\times20\times20\ \mathrm{cm}^3$) for 0, 24, 48 or 72 h. Males were grouped to simulate intense sexual competition in gregarious conditions and to avoid a drop in population density. Each animal was tested only once, but three to four males from the same group of 10 were used in simultaneous experiments. During 120 min of observation, all mating attempts by the test males were recorded by an observer. If a male jumped from a short distance onto the couple, this event was classified as an attack (the same behaviour is shown by males when mounting an unguarded female). The guarding male regularly attempts to kick off the assailant. However, if the attacking male was able to evict the guarding male from the female after a short struggle, this was classified as a takeover event and the experiment was terminated. Females always remained passive or tried to escape from this scramble, never supporting one or the other male.

One-way ANOVA with *post hoc* test for multiple comparisons and *t*-test procedures of the SPSS software package (v. 13.0.1, SPSS, Inc.) were used to test for significant differences in the number of mating attempts between males deprived for different times. All groups were tested for homogeneity of the variances using a Levene test prior to analysis and the appropriate procedure was selected. In case of heterogeneous variances, the degrees of freedom were corrected by SPSS resulting in a fraction value. Observed frequencies of mating attempts or takeovers were analysed by a χ^2-test using the exact tests module of the SPSS software package.

3. RESULTS

Males started to ignore the CIP of the guarding competitor after 24 h of female deprivation (table 1). All males that had not met a female for 72 h disregarded the pheromone and tried to takeover the receptive female. If males started to attack the couple, their pertinacity in trying to usurp the female (the number of attempts they made) depended on the deprivation time (ANOVA, $F(2, 35)=9.169$, $p=0.001$; only data of unsuccessful males were included in this analysis because a takeover renders further attempts superfluous). However, only the 72 h female-deprived males attacked significantly more often than the males of the other groups within the observation period

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Table 1. Takeover attempts by female-deprived *S. gregaria* males.

		males attacking			success rate (%)	
deprivation (h)	tests	(%)	attacks[a] (mean ± s.d.)	number of takeovers[b]	attacks	males
0	19	0	—	0	—	—
24	19	42	2.1 ± 1.77	1	4.7	12.5
48	19	63	2.6 ± 2.51	5	19.2	41.6
72	18	100	9.1 ± 5.43	4	2.9	22.2

[a] Only data from males that attacked the couple but did not usurp the female; see text.
[b] Number of successful males per deprivation level.

(Tamhane test, both $p<0.004$), while the other two groups did not differ (Tamhane test, $p=0.978$). The attacks were not evenly distributed over the observation time with more attacks undertaken within the first observation hour (figure 1; number of attempts standardized for 38 males to compensate for a declining number of competing males owing to successful takeovers, $\chi^2=44.180$, $p<0.001$).

The observed overall takeover rate was 5.4% (10 out of 185 attempts were successful). Takeovers were evenly distributed over time (figure 1; for statistical analysis grouped in intervals of 30 min, $\chi^2=3.600$, $p=0.431$). Successful males took 2.4 ± 1.71 (max. 6) attempts to usurp the female (figure 2) independent of the deprivation time (males deprived for 48 h or less: 2.3 ± 2.16, males deprived for 72 h: 2.5 ± 1.00; $t=-0.142$, d.f. = 8, $p=0.890$). There was no difference in the time needed to takeover the female between these two groups ($T=-1.225$, d.f. = 8, $p=0.255$; total mean 46 ± 34 min). It is noteworthy that unsuccessful males did not attack more than successful males if they had been deprived for 48 h or less (2.4 ± 2.10, $t=0.023$, d.f. = 18, $p=0.982$), while longer deprived unsuccessful males tried more often to usurp the female than the successful ones (9.1 ± 5.43, $t=4.326$, d.f. = 15.332, $p=0.001$). Consequently, the success rate per takeover attempt differs between deprivation times (table 1; $\chi^2=10.841$, $p=0.011$). The success rate per attacking male (total 26.3%) did not differ significantly between deprivation times ($\chi^2=1.769$, $p=0.461$).

4. DISCUSSION

The CIP, PAN, is not able to completely inhibit the rival males from attempting to oust the resident male from a couple. Whereas PAN serves to inform the recipient that the sender is a mature male, the pheromone does not hide the presence of the receptive female. Locust males are able to distinguish between other mature males and couples. They do not try to mount single males (but they do so with immature or isolated males, both of which lack PAN; personal observations).

Males start to ignore PAN when they are female deprived for one day. After three days spent without a potential mate, all males are willing to accept lower quality females (i.e. already inseminated; costs associated with fighting). Moreover, males attack couples with increasing intensity as deprivation time lengthens.

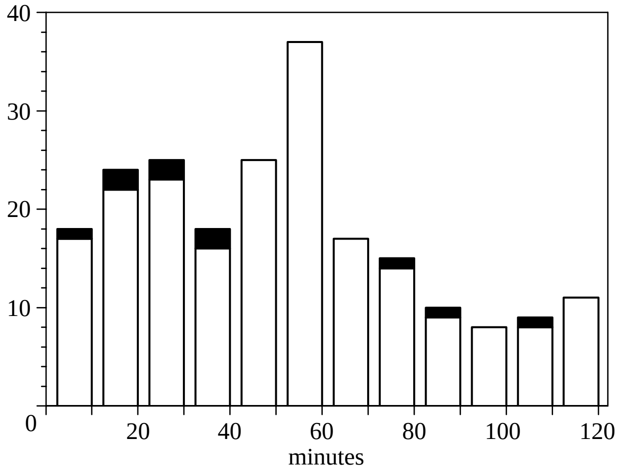

Figure 1. Frequency distribution of takeover attempts of female-deprived *S. gregaria* males in dependence of observation time. Successful attempts are marked by solid fill pattern.

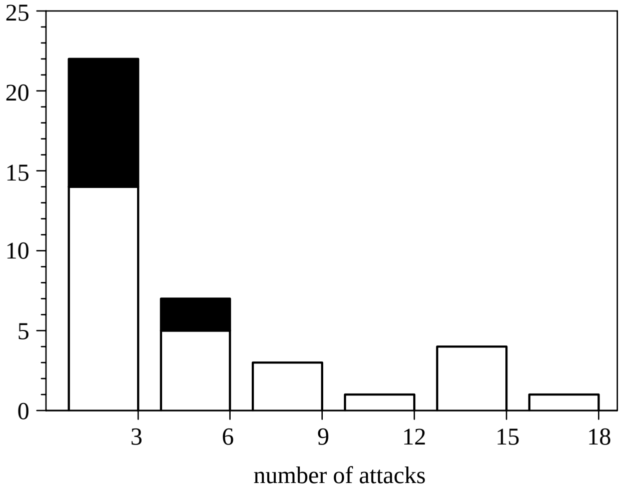

Figure 2. Frequency distribution of the number of takeover attempts of female-deprived *S. gregaria* males within 120 min. Successful attempts are marked by solid fill pattern.

Within the first two days of deprivation, males limit their attacks to a few attempts. They did not attack more often than usually necessary to succeed. After having been deprived for more than 48 h, males attempt to takeover the female more often without having more success. This last result points to the fact that the outcome of a fight over a mate depends on certain traits of the struggling males that are still unknown. Either an assailant is able to oust the male and succeeds within a few attempts or he is not. His chances do not increase if he tries more often.

My results indicate that PAN can be considered an analogous signal to the host-marking pheromones (HMP) of phytophagous insects and parasitoids (Nufio & Papaj 2001). In both the cases, the occupation of a resource is announced and the recipient decides whether or not to respond. Females with a heavy egg load have been found to ignore the HMP (Roitberg & Prokopy 1983; van Alphen & Visser 1990; van Randen & Roitberg 1996), in much the same manner as female-deprived male desert locusts ignore PAN. Notably, there are no reports so far that male-donated anti-aphrodisiacs have been ignored. Females using male-donated anti-aphrodisiacs are monandrous and refuse to re-mate at least for a certain time (Simmons 2001). However, in *S. gregaria*, females accept several matings in a row and males guard actively to ensure paternity, though (Seidelmann & Ferenz 2002).

The decision of a male to regard or to disregard the CIP of a rival depends on the expected fitness gain in terms of fertilized eggs per time. Given that the mean clutch size is 72 eggs and sperm precedence of the last male is 67.3% (H. Koch 2005, unpublished results from the *S. gregaria* culture used for the experiments), it can be estimated that a male choosing to attack mating pairs, in which the male has already transferred sperm, will sire only 13 eggs on an average (i.e. 0.263 success rate × 0.673 sperm precedence × 72 eggs). It will be worth it doing this if the fitness gain from finding an unguarded receptive female is worse than 13 eggs per time necessary to takeover (0.77 h) and guard the usurped female (caged *S. gregaria* males guard on an average 9.08 h; F. Neugebauer 2004, unpublished results). The search time needed to find a vacant mate determines whether it pays to regard or to disregard the pheromone signal. Under the conditions outlined below, a balanced fitness gain from both decisions can be expected at a search time of 45.47 h. Although only females with mature eggs accept males for copulation and thus the operational sex ratio is male biased, males in high-density populations contact many mature females. Therefore, the search time depends mainly on the deprivation time given by the experimental design. For search times longer than 46 h, more and finally all males can be expected to disregard the CIP. This fits the data of my experiment pretty well. It will be interesting to establish under what circumstances such conditions occur in the field.

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