

Flight Initiation by Male *Rhodnius prolixus* is Promoted by Female Odors

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Abstract Several triatomine bug species utilize chemical cues for sexual communication. We tested whether female or male *Rhodnius prolixus*, a vector of Chagas disease, produce volatile chemicals that elicit flight responses from conspecifics, and then isolated the source of the chemical. Males confronted with an airstream containing female odors showed a significantly greater take-off frequency compared to a blank airstream or an airstream with male odors. In contrast, females exhibited similar take-off frequencies to male or female odor as to a clean airstream. Occlusion of female metasternal glands with paraffin wax resulted in a significant decrease in male take-off frequency compared to that of intact females. Additionally, excised female metasternal glands elicited a similar take-off frequency from males as did intact females, both significantly greater than the take-off frequency to clean air. These results show that *R. prolixus* females release a pheromone from their metasternal glands that causes upwind flight in conspecific males.

Key Words *Rhodnius prolixus* · Flight · Sexual behavior · Communication · Metasternal glands · Pheromones · Chagas disease · Trypanosoma · Hemiptera · Reduviidae · Triatominae

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Introduction

Rhodnius prolixus Stål (Hemiptera: Reduviidae: subfamily Triatominae) transmits *Trypanosoma cruzi*, the etiological agent of Chagas disease, in northern South America and parts of Central America. In Latin America, Chagas disease afflicts 15 million people, with roughly a further 90 million at risk from potential transmission.

Triatomines are capable of sustained flight, dispersing over considerable distances (Gringorten and Friend 1979; Schweigmann et al. 1988). Flight is influenced by temperature and nutritional state of the insect (Lehane et al. 1992; Gurevitz et al. 2006). Adults are attracted strongly to light sources (Noireau and Dujardin 2001; Minoli and Lazzari 2006).

It is known that male triatomine bugs are attracted to and aggregate around mating pairs of adults (Baldwin et al. 1971; Manrique and Lazzari 1995). Triatomine bugs have paired Brindley's (BGs) and metasternal glands (MGs) that emit volatile compounds. The compounds emitted from the MGs, in particular, appear to mediate triatomine sexual communication (Crespo and Manrique 2007; Pontes et al. 2008). Pontes et al. (2008) demonstrated that female *R. prolixus* emit MG odors at dusk and in greater quantities than males.

We evaluated whether male or female odor added to an airstream promotes an increase in flight initiation or oriented take-off in adult *R. prolixus*. Also, we demonstrated that the source of the odor responsible for this effect are the female MGs.

Methods and Materials

Rhodnius prolixus were obtained from the Servicio Nacional de Chagas, Argentina, and reared in the laboratory

at $26\pm 1^\circ\text{C}$, 30–50% RH, 12:12 h L:D, with live hens as a blood meal source. Hens were handled according to the biosafety rules from the Servicio de Higiene y Seguridad, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. Bugs were sexed before final ecdysis, and the two sexes were kept separately until use. One to 2-months-old virgin adults, starved for 20 ± 5 d, were used in the experiments.

We used a cage constructed of voile fabric (based on Minoli and Lazzari 2006), with a circular acrylic arena (15 cm diam. \times 5 cm high) hung 1 m above the center of the floor. A vertical wooden rod (1.5 cm diam. \times 15 cm high), fixed in the center of the arena, functioned as a platform for flight take-off. Bug movement was recorded with an infrared video camera (Videoman, JCC-300, Korea), with an infrared LED light source illuminating the platform. The video monitor was divided into eight 45° sectors, centered on the image of the top of the take-off platform, allowing determination of the angular direction of take-off. A Vaseline[®] film, applied to the interior surface, allowed only flying insects to leave the arena. A fan fixed inside an 85-cm-long PVC tube (12 cm i.d.) generated an airstream of 12 ± 0.9 cm sec^{-1} (measured with a Testo 405-V1 anemometer, Germany) at the take-off platform. Insects used as odor sources were placed inside mesh-covered, open-ended flasks that permitted airflow. Groups of 20 males or females (four replicates per treatment) were released onto the arena at the start of the scotophase and left for 8 hr. We recorded the number of take-offs and their angular direction.

In the first experiment, we tested the responses of males and females, separately, to: a) clean air, b) air with odors from females, and c) air with odors from males. Ten insects per flask were used in all treatments using insects as odor sources. Next, we occluded the orifices of female glands with paraffin wax (Crespo and Manrique 2007) 24 hr prior to testing the responses of males to the following treatments: a) BG-occluded females, b) MG-occluded females, c) MG- and BG-occluded females, and d) sham females that had wax applied between the prosternum and metasternum that did not occlude the glands. Finally the take-off frequency and orientation of males was also tested to airstreams with: a) excised female MGs (ten glands), b) intact females, and c) control consisting of ten pieces of tissue and cuticle from the coxae of the first legs of females.

Take-off frequency data were analyzed by linear parametric statistics, after normality (P-Plot) and homoscedasticity (Levene's test) were verified. A two-way ANOVA, assuming fixed factors (gender and stimulus type), or a one-way ANOVA was applied, as appropriate. A significant ANOVA was followed by a *post-hoc* comparison (Tukey test). The MGs odor data set did not meet normality assumptions; therefore, data were log-transformed. Take-off

frequencies presented in the results section are means (\pm SEM) from four replicates.

Results and Discussion

Typically, responsive insects climbed the take-off platform, moved their antennae, and took flight. There was a significant interaction (two-way ANOVA, $F_{2,18}=12.63$, $P<0.001$) between gender and stimulus type, indicating that males and females responded differently to stimuli. Male take-off frequency varied with stimulus (Simple effect, $F_{(2,18)}=11.94$, $P<0.001$), with the greatest response being to female odor. There were no significant differences in responses of females to any of the odor treatments (Fig. 1a).

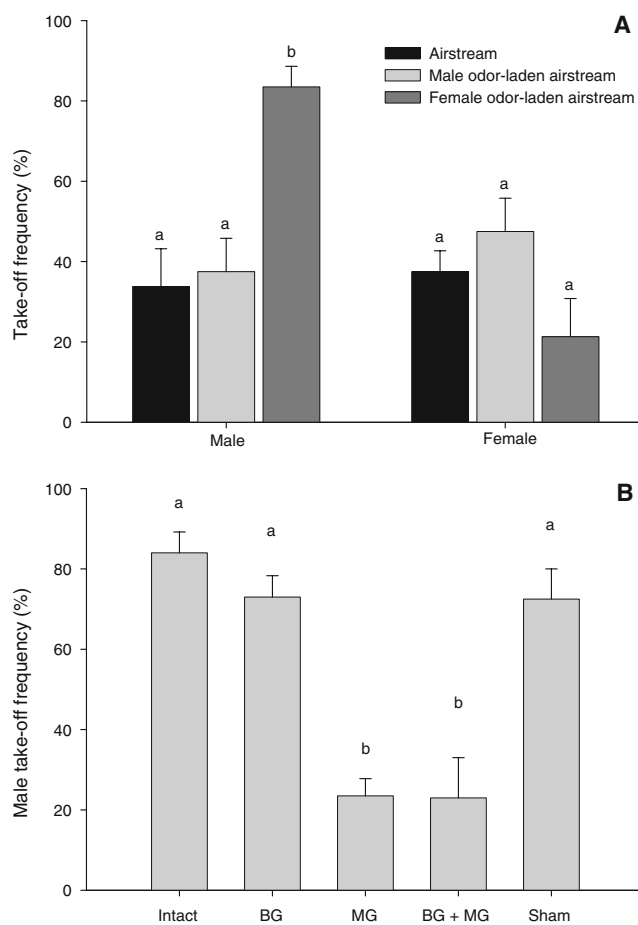


Fig. 1 Take-off frequencies (mean \pm SEM) of: **a** *Rhodnius prolixus* males or females to airstreams with intact males, intact females or clean air (control), and **b** *R. prolixus* males to various odor-laden airstreams; Intact = intact females, BG = females with Brindley glands occluded with paraffin wax, MG = females with metasternal glands occluded, MG+BG = females with metasternal and Brindley glands occluded, Sham = females that had paraffin applied to the cuticle without occluding any glands. Different letters atop bars denote statistically different means (Tukey, $P<0.05$). Each treatment consisted of four replicates

In the second experiment, flight frequencies showed a significant (one-way ANOVA, $F_{(4,15)}=19.95$, $P<0.001$) effect of occlusion treatment, with females with occluded MG or MG+BG eliciting significantly lower ($P<0.05$) take-off responses from males than did intact, sham-operated, or BG-occluded, females (these three treatments elicited similar take-off frequencies; Fig. 1b). In the final experiment, male take-off frequency was different depending on treatment (one-way ANOVA, $F_{(2,9)}=13.75$, $P=0.002$). Excised glands elicited a similar take-off frequency as intact females ($60\pm 7\%$ vs. $84\pm 5\%$; $P>0.05$, Tukey test). However, odors from control tissues elicited a lower male take-off frequency compared to odors from intact females ($34\pm 7\%$ vs. $84\pm 5\%$; $P<0.05$) or from excised MGs ($34\pm 7\%$ vs. $60\pm 7\%$; $P<0.05$, Tukey test).

The take-off direction of insects had a non-uniform distribution for all treatments tested. Insects tended to orient toward the direction of the airstream, regardless of odor.

The present work demonstrates that the flight of adult male *R. prolixus* can be triggered by volatile chemical cues released by conspecific females. In contrast, females did not show significant flight responses to male chemical cues. The source of the volatiles that elicit male flight appear to be the MGs of females, because when this gland was occluded with paraffin wax, the flight responses of males decreased significantly. In contrast, occlusion of another gland of females, the BG, or sham operation, did not affect flight responses of males. This sex-specific response suggests that this behavior is part of a mate location mechanism involving a sex pheromone emitted by females (Pontes et al. 2008).

Now that we have established that female *R. prolixus* produce a pheromone that elicits flight from males, chemical identification can proceed. Indeed, the chemical (s) may already have been identified in the study of Pontes et al. (2008). Identification of the pheromone will allow us to determine whether male behavior can be manipulated for controlling vectors of Chagas disease.

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