Previous Motor Experience Enhances Courtship Behavior in Male Cricket *Gryllus bimaculatus*

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Abstract In crickets *Gryllus bimaculatus*, flight has been shown to be able to promote aggressive encounters between males and to suppress escape behavior. The aim of this study was to examine the influence of flight on male behavior in male–female interactions. We found that flown males demonstrate enhanced courtship behavior. The latency of calling song was significantly shorter, while the relative total duration of singing as well as the duration of singing episodes longer in flown males than in the control. Mating rate was also significantly higher in the experimental group containing flown males. The results suggest that, in addition to previously reported effects on aggressiveness and escape, flying has a profound accelerating effect on male courtship behavior.

Keywords Insect · flight · motor experience · sexual behavior

Introduction

The field cricket *Gryllus bimaculatus* has been extensively used as a model system for studying behavioural and neuroethological aspects of insect aggressive encounters (Adamo and Hoy 1995; Hofmann and Schildberger 2001), female choice (Simmons 1986, 1988a, b) and mating (Adamo and Hoy 1994; Sakai et al. 1995; Tregenza and Wedell 1997). Recently, the discovery of an unusual behavioral occurrence has brought renewed interest to this animal. It was found that a seemingly unrelated motor activity, flying, dramatically alters aggressive behavior in the male crickets. The immediate reset of aggressiveness by flying was first observed in the losers, who normally avoid winners for hours after losing fights to them

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(Hofmann 1997; Hofmann and Stevenson 2000). Studies carried out later showed that experience of flying does not only restores aggressiveness in losers, it also dramatically enhances fights between initially aggressive males and suppress their escape behavior (Stevenson et al. 2005). Biological sense of these effects remains however obscure.

It has been suggested that energetically costly flights are paid off in intruders by increasing the chances of winning key resources in a new territory (Stevenson et al. 2005). If flight causes a behavioral state, which is similar to that of a winner, it might be expected to enhance courtship behavior in male–female interactions. It is known, that winners engage in aggressive behavior when they come across a conspecific male, but start courtship behavior when they encounter a conspecific female (Alexander 1961). Moreover, there is a positive relationship between the intensity of courtship display and competitive ability in male crickets. Fight winners call females more frequently than losers and have a greater mating success (Simmons 1986).

On the other hand, calling attracts not only females, but also conspecific males, predators and parasites (Gray and Cade 1999). This behavior does not seem to be as advantageous as aggression could be on a new territory where the risk of predation and competition is initially unknown. An alternative hypothesis may suggest that a need to compensate for the loss of energy during flight causes hunger behavior in flown crickets. Starvation is known to promote interspecific aggression and even cannibalism in *G. bimaculatus* (Adamo and Hoy 1995). Hungry crickets, however, unlike winners of male competition, are aggressive in both intra- and inter-sexual interactions (Adamo and Hoy 1995). If flight-induced interspecific aggression of crickets is a hunger symptom, flown males might be expected to be aggressive towards conspecifics of both sexes.

It is therefore reasonable to check how forced flying in male crickets affects male–female relationships to better understand the nature of flight phenomenon. We addressed the following questions: (1) do flown males attack or court females, and (2) does flight affect the intensity of aggression and/or courtship behavior? Previous works on *G. bimaculatus* (Stevenson et al. 2005; Adamo and Hoy 1994) provide good experimental paradigms for quantification of both aggressive and courtship behavior in this animal.

Materials and Methods

The crickets *G. bimaculatus*, 2–3 weeks after the last imaginal moult, from a breeding colony provided by the Entomological Department of Moscow Zoo were kept isolated for at least 24 h before the experiment, with food and water ad libitum (25°C, 13–11 h light–dark cycle). That period of isolation was expected to eliminate the differences between the animals related to their social and sexual experience (Hofmann 1997; Sakai et al. 1995). Females were provided with a small dish containing damp sand in which to oviposit. The males and females were randomly paired up.

The experimental procedures utilised to identify the influences of flying on cricket behavior were essentially those utilised earlier (Stevenson et al. 2005). Cricket was glued to a holder, and flight behavior was triggered by suspending the animal for 3 min in an air stream ($N=30$). The controls (non-flown males, $N=37$)

were handled and kept for 3 min in cages near the air stream. The animals were then placed for 5 min in separate compartments of a 20 cm×12 cm×12 cm transparent cage. Interaction was allowed by removing a divider. Behaviors in the arena were observed for a 5-min period after initial physical contact between the animals was made. Behaviors were observed directly and scored using an event recorder. A one-character code was assigned to each of the different components of behavior studied in the experiments, and the respective key on a computer keyboard was pressed to indicate each start and end of a behavioral sequence. The code together with a time stamp (resolution 0.1 s) was stored in files for subsequent analysis. The entirety of the experiment was videotaped using a Sony CCD-FX400E and tapes used to test the personal error of the observer (found to be about ±0.5 s) and confirm questionable live scoring.

Within each pair, we checked to see whether the male engaged in aggressive or courtship behavior, whether it had sung and, if yes, then what type of singing it did (calling or courtship, for review see Boake 1983; Bennet-Clark 1989), the start and end of each singing episode, and whether copulation occurred. Interaction was regarded as aggressive if one or both animals spread mandibles (level 3 and higher according to Hofmann 1997).

We calculated the latency of singing (time between the first physical contact with a female and start of singing) and the total duration of singing (time spent singing). For final analysis, we used a relative total duration of singing estimated as percent of the total time spent with a female until copulation or the end of observation. We calculated the duration and number of calling and courtship songs. Crickets can switch from one song to another without a break (pause no greater than 0.5 s, comparable with interval between calling bouts). We monitored the duration of each period of unceasing singing and total number of these periods.

ANOVA (for parametric) and Kruskall–Wallis ANOVA (for non-parametric data) were used for testing the significance of the observed differences in latency and duration of singing. Fisher's test was applied to test the difference between the control and experimental group in occurrence of fight, courtship and copulation.

Results

Aggressive interactions were observed in 4 from 30 pairs containing flown males (13.3%), and in five from 37 pairs (13.5%) in the control. The main stages of male–female conflict escalation were found to be similar to those described for intrasexual fights: between two males (Hofmann 1997; Stevenson et al. 2005) or between two females (Adamo and Hoy 1995; Dyakonova et al. 2000, 2002).

Flight markedly enhanced courtship behaviour in the males as they sang more calling and/or courtship songs (Fig. 1a). Courtship occurred in 26 from 30 pairs containing flown males, whereas in the control it was only in 15 from 37 pairs. In some control pairs (*n*=17), neither aggressive nor courtship behavior occurred, and animals, after the first physical contact, avoided each other or behaved independently, walked and scanned the territory.

Latency of singing was significantly shorter in the experimental group (Fig. 2). Some flown males started to call even before they contacted a female in the arena,

Fig. 1 Effect of flying on occurrence of singing (**a**) and copulation (**b**) in female–male pairs. Charts represent the percentage of pairs in which these behaviors were observed.
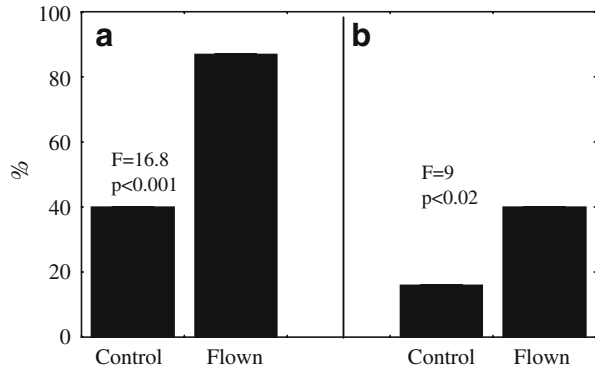
and one cricket, being suspended in the holder, switched from flying to calling when the fan was turned off.

Relative total duration of singing was significantly higher in flown males than in the control (Fig. 3a). The number of periods of unceasing singing did not differ between flown and control males ($n=3.2\pm0.5$ and 3.9 ± 1, $p=0.6$), while the mean duration of single period of singing was significantly increased (Fig. 3b).

In both groups, the periods of unceasing singing were represented mainly by song of a one type, either calling or courtship. Sometimes, however, calling and courtship singing followed each other without a break. In such cases, a period of unceasing singing consisted of alternate episodes of calling and courtship singing. Flying significantly increased occurrence of these composed periods of unceasing singing (23% versus 10.4% in the control, $F=3.27$, $p<0.05$). The mean number of alternate calling/courtship songs within a single period of unceasing singing was 1.3 ± 0.1 in the control and 3.5 ± 0.7 in the experimental group, $F=6.2$, $p<0.02$. The maximal number of songs within a single period reached 25 in the experimental group, while in the control it was only 6.

The ratio between calling and courtship singing did not differ between the experimental and the control group: percentage of calling was $61\pm11\%$ and $69\pm8\%$, respectively, $p>0.5$. Significant increase in the duration of songs of both types, calling and courtship was observed in the experimental group containing flown males (Fig. 4).

Fig. 2 Effect of flying on latency of singing. Mean with standard errors. *H* Kruskall ANOVA test.
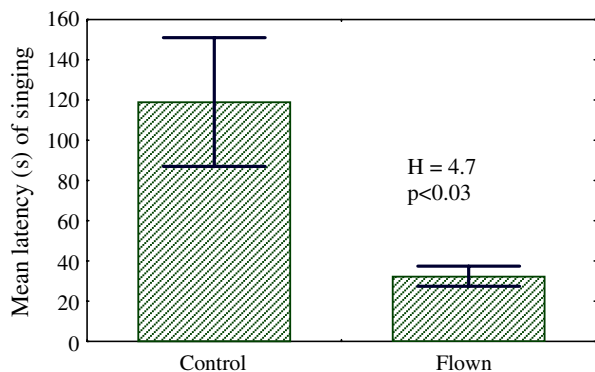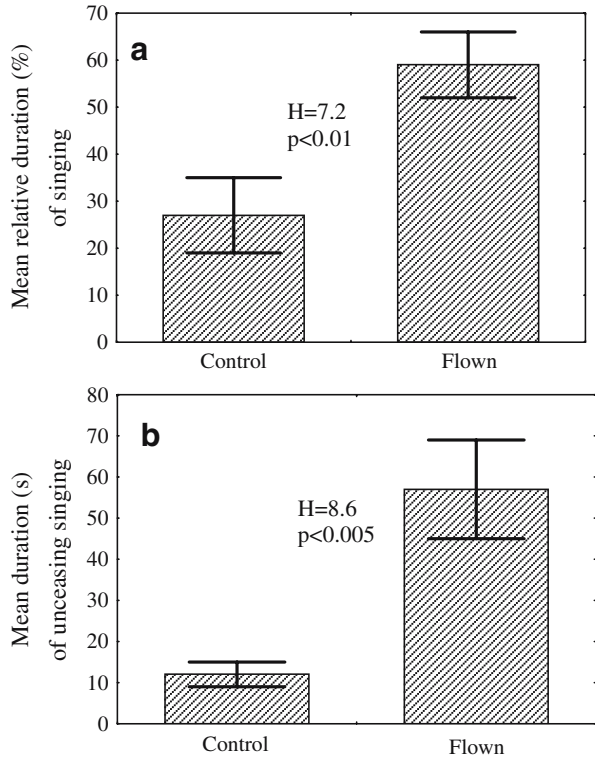
Fig. 3 Effect of flying on relative total duration of singing (a) and duration of unceasing singing (b). Means with standard errors. *H* Kruskall ANOVA test.

Copulation was significantly more frequent in the experimental group than in the control (Fig. 1b). It occurred in 12 from 30 pairs containing flown males, whereas in the control it was in 6 from 37 pairs only. The increase in the mating rate was due to higher number of singing males in the experimental group. There is no significant difference in the occurrence of copulation if non-singing males are excluded from the analysis: 12 from 26 pairs with singing males in the experimental group, and 6 from 15 in the control.

Fig. 4 Effects of flying on duration of calling (a) and courtship (b) songs. Means with standard errors. *H* Kruskall ANOVA test.
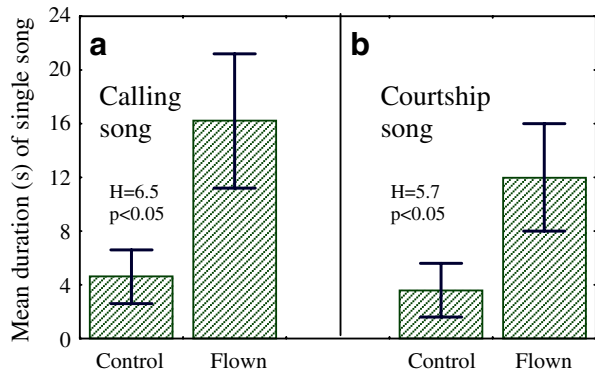
Discussion

Flight does not Cause Aggression in Males towards Females in Male–Female Interactions

Generally, in male crickets, the choice between fighting and courting is known to be strongly depending on the sex of the partner with cuticular pheromones playing a key role in sex recognition (Tregenza and Wedell 1997, Nagamoto et al. 2005). However, certain physiological states, such as starvation or sexual refractoriness, may promote aggression in male–female interactions (Sakai et al. 1995; Adamo and Hoy 1995). Unlike these conditions, experience of flying which is known to strongly enhance aggressive behavior of males towards males (Hofmann and Stevenson 2000; Stevenson et al. 2005), did not enhance aggressive behavior of males towards females in our experiments. Frequency of aggressive interactions was low (about 13%) and almost equal in the control and experimental groups. Flown males therefore do not seem to defy the general rule "fight male, court female". Courtship was a prevalent behavior in the experimental group.

Flying Promotes Courtship Behavior in Males

It has been known that previous behavioral experience can affect sexual behavior in crickets. Density of population (Alexander 1961; Simmons 1986), outcome of male competition (Simmons 1986; Adamo and Hoy 1994), mating experience (Sakai et al. 1995), starvation (Adamo and Hoy 1995) influence the intensity of the courtship display. Previous motor experience has also been shown to enhance the activity of the pattern generator for copulatory actions in the male *G. bimaculatus* (Matsumoto and Sakai 2001). Here, we demonstrate for the first time that seemingly unrelated motor activity, flying, has a profound effect on calling and courtship singing in males *G. bimaculatus*.

In our experiments, a flown male when encountered a female started to trill earlier and spent more time singing. Calling/courtship songs were produced in many more pairs containing flown males than in the control. Flying prolonged the duration of periods of unceasing singing as well as the duration of single (calling or courtship) songs.

Calling and courtship songs in *G. bimaculatus* differ in their function and energetics. Calling song is a loud distant signal attracting females (Alexander 1961; Loher 1989; Simmons 1988a). Soft courtship singing usually occurs when female is near by a male (Alexander 1961; Loher 1989; Tregenza and Wedell 1997; Nagamoto et al. 2005). In our experiments, flight had no discriminating effect on type of singing, the ratio between calling and courtship singing was similar in the control and in the experimental group. This indicates that flight enhances sexual motivation in crickets, but does not change principal rules in the organization of their courtship behavior.

Copulation Occurs More Frequently in Flown Crickets

Like many insect species, female crickets usually choose mates based on male size (Simmons 1988a), fighting abilities (Savage et al. 2005; Kortet and Hedrick, 2005),

scent (Kortet and Hedrick 2005) and intensity of the courtship display (Simmons 1988b). In agreement with the hypothesis of honest signaling in sexual selection (Zahavi 1975), courtship display is indicative of male social status and even immunocompetence. In *G. bimaculatus*, a positive relationship had been found between the courtship display and competitive ability (Simmons 1988b), and, more recently, a correlation between the intensity of courtship and immunocompetence was demonstrated (Rantala and Kortet 2003, 2004).

In our experiments, the intensity of courtship behavior was impressively enhanced by previous experience of flying, and, not unexpectedly, the copulation occurred more often in pairs containing a flown male. The latter effect depended on the former: no significant difference in mating rate was seen between control and experimental groups if non-singing males were excluded from the analysis. Similarly, in the house cricket *Acheta domesticus*, "shaking" males in clasped hands was reported to have no effect on female preference between singing flown and singing non-flown males (Savage et al. 2005).

In experimental conditions, crickets, housed in a small arena for 1 h before the interaction, court and mate either within a 15-min period (preferably) or never (Simmons 1986; Adamo and Hoy 1994). In our experiments, the crickets had a comparatively short period of housing (5 min) in the arena prior to interaction, and interaction was only allowed for 5 min. In addition, control animals were handled and kept near the fan before the interaction. This may explain a lower occurrence of mating in the control (non-flown) pairs in our experiments than in earlier works (Simmons 1986; Adamo and Hoy 1994; Savage et al. 2005).

After copulation, females *G. bimaculatus* can remove the spermatophore of "undesirable males" (Simmons 1986). We did not check here the effect of flying on probability of spermatophore rejection. *G. bimaculatus* females are polyandrous, and female sperm choice may additionally affect male reproductive success (Tregenza and Wedell 2002). Nevertheless, it seems likely that, by increasing courtship behavior, flight may enhance male reproductive chances.

Flying Causes a Behavioral State Similar to that of a Winner

It has previously been shown that other types of motor activity, stress, and being kept in the air stream are irrelevant to the effects of flight on cricket behavior (Stevenson et al. 2005). Flight has been suggested to create specific behavioral state in crickets by causing a constellation of neurohormonal, metabolical and behavioral changes (Adamo et al. 1995). Our results confirm this idea and suggest that the previously described activation of aggressiveness towards rivals (Hofmann and Stevenson 2000; Stevenson et al. 2005), inhibition of escape behavior (Stevenson et al. 2000) and shown here enhancement of courtship are parts of complex changes in the behavioral state of male crickets after flight. All these effects of flying appear to be well co-ordinated. In fact, they all are the features of a winner (Alexander 1961; Simmons 1986; Hofmann 1997; Dyakonova et al. 1999). It appears therefore, that, in male crickets, flying causes a behavioral state, which is similar or identical to that of a winner. Or, in other words, flight enhances male competitive ability and reproductive chances by reducing escape behavior, on the one hand, and by increasing aggressive and courtship behavior, on the other.

Flying behavior is an example of within and between habitat dispersal in the field crickets (Walker 1986). *G. bimaculatus* are capable of flight during the first days after the last imaginal moult. In adult animals, some of the flight muscles may degrade (Shiga et al. 1991; Zheverikhin 2002) and flight behavior can only be caused by a strong airflow. It remains to be elucidated whether the observed effect of flight is of biological significance in *G. bimaculatus* under natural conditions.

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