The effects of female cricket pheromones on the aggression of male *Acheta domesticus* crickets

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Abstract

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The purpose of this study was to address the relationship between the presence of female cricket pheromones and the male crickets' competitive and aggressive behaviors. A repeated measures design was used to test the effects of three different conditions on the fighting behavior of eight pairs of crickets of similar size. The three conditions included a fighting arena with the presence of no female pheromones, pheromones from one female cricket, and pheromones from three different female crickets. The levels of aggression exhibited by the male crickets were measured by recording the time it took for the crickets to engage in fighting behavior and the maximum level of aggression that the pair reached before establishing a dominant winner.

The results obtained in this study supported our hypothesis that when exposed to an environment containing pheromones from one female cricket, the male crickets would be more aggressive due to their competitive instincts in finding a mate. The male crickets showed the least amount of aggression when placed together in an environment containing pheromones from three different female crickets, indicating a decrease in their need to be competitive since the resource of mates was large and sufficient. When in the presence of no female pheromones, the male crickets exhibited normal aggressive behavior.

Introduction

Crickets have been an efficient source for researchers to study the aggressive behavior of animals for years. Male crickets are known to exhibit aggressive behavior towards one another when competing for shelter and females, and this behavior is conveniently observable in the lab setting. This experiment focused on the role of pheromones provided by female crickets in altering the aggressive behavior of male crickets. The purpose of this study was to examine if the presence of female cricket pheromones before exposure of two male crickets to one another would cause the crickets to exhibit more aggressive behavior due to an increase in competition for the female. Pheromones are chemicals produced and released by animals that cause a behavioral effect on other insects or animals [10]. A study conducted by Tom Tregenza and Nina Wedell showed evidence of a link between male cricket aggression of the field cricket *Gryllus bimaculatus* and a communication channel consisting of cuticular pheromones. Support for this research showed that males would exhibit mating behavior on an anaesthetized female but not one coated in plastic [15]. This research shows that the presence of pheromones released by the female may be a factor in mating behavior among male crickets. Much research has been done to provide explanations for the aggressive behavior of crickets to be generalized to that of other animals. According to game theoretical models, the aggressive behavior of two male crickets engaged in a contest is highly associated with an initial assessment of relative fighting ability and motivation [2]. Other studies have shown that a “fight and flight” response for crickets that lose a fight can restore their aggression and motivation due to biogenic amines [13].

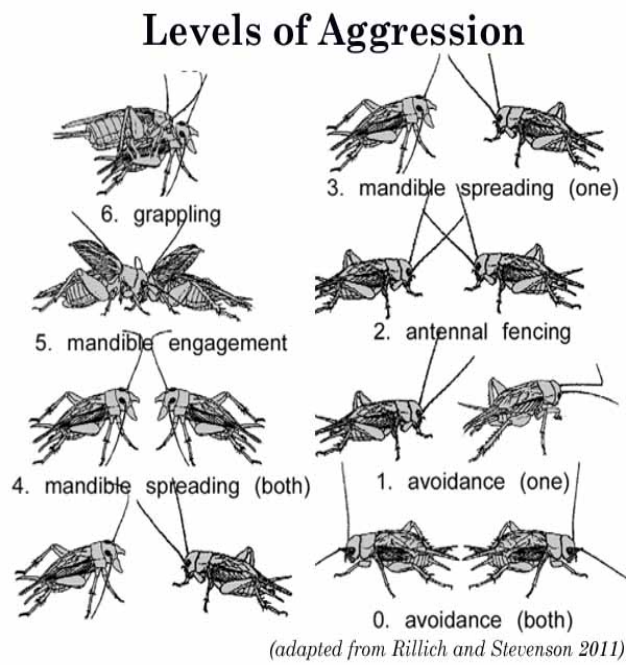
Other evidence emphasizes the traditional sex roles of crickets, where the males tend to compete in an aggressive manner for access to the female crickets [12]. In support of female crickets as a factor in male cricket aggression, the presence of females has been found to modify aggressive motivation by triggering the release of the aminergic neuromodulator octopamine, which can increase aggression in crickets [11]. A study by Killian and Allen showed that a male cricket's motivation to acquire dominance against another male has a positive relation to mating with a female [7]. Our hypothesis was that when male *Acheta domesticus* crickets were exposed to an area containing pheromones from one female cricket, they would exhibit significantly more aggressive behavior due to mating competition than when exposed to an area with no pheromones or with pheromones from more than one female cricket.

Methods and Materials

To test the hypothesis, a repeated-measures experimental design was used in which eight pairs of *Acheta domesticus* crickets were exposed to three different conditions and measured on their level of aggression. We first obtained sixteen *Acheta domesticus* male crickets that had been reared by being isolated for seven days in order to promote aggression. The basic procedure used for all three conditions included obtaining two male crickets of similar size and inducing aggression through a flight response by tossing them in the air and shaking them in our hands. This strategy was used to increase aggression because of evidence from studies that show aggressiveness in crickets can by restored by activating their motor program through flying [5]. The two crickets of similar

size were then placed in a fighting arena at the same time, where their behavior was recorded for three minutes. The crickets were then taken out, and the procedure was repeated again using the other two conditions and the same pair of male crickets. For the first condition, the pair of male crickets was placed in the fighting arena for two minutes, with no female crickets placed before them. The second condition involved placing one female in the fighting arena for one minute, removing her, and placing the male crickets in the arena for two minutes then removing them. The third condition involved this same procedure but with three female crickets. This procedure was repeated for eight trials, using a total of sixteen male crickets and five female crickets. In order to measure the level of aggression displayed by the crickets under each condition, we recorded the time in seconds from the initial exposure of the male crickets to one another in the arena and the time of first indication of a fight. These indications included antennal fencing, mandible spreading or engagement, and wrestling. We then observed the winner of the fight by a rivalry song and recorded the maximum level of aggression that the crickets reached before indication of a winner. The levels of aggression were numbered from zero to six in the order of mutual avoidance (0), pre-established dominance (1), antennal fencing (2), unilateral mandible spreading (3), bilateral mandible spreading (4), mandible engagement (5), and wrestling (6).

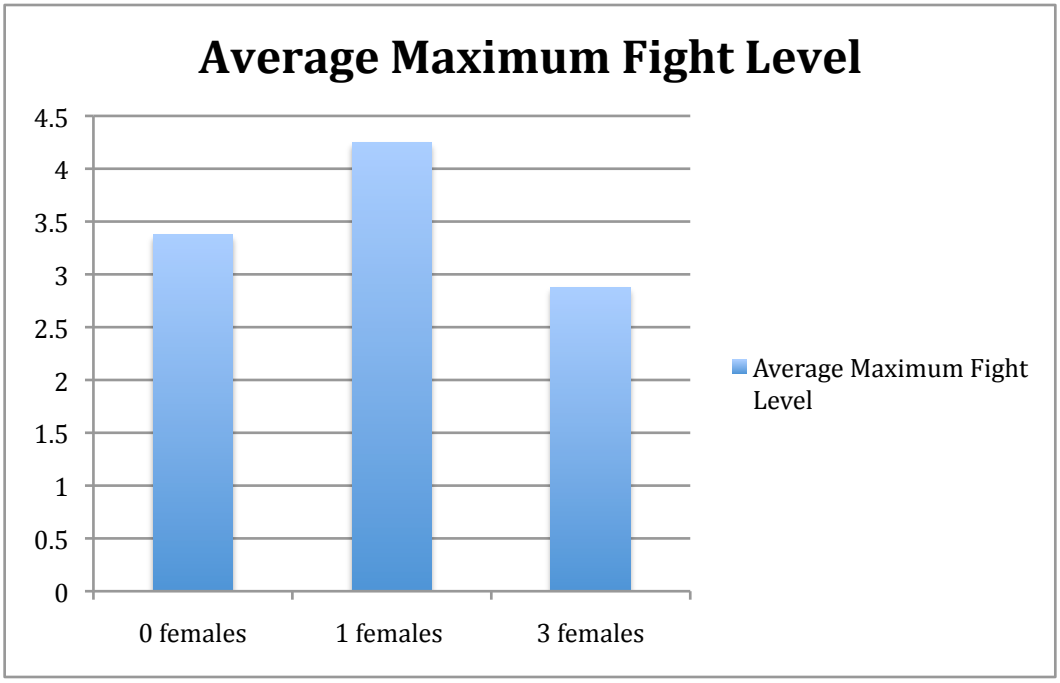
The figure to the right illustrates the levels of aggression of male crickets.



The fighting arena was cleaned thoroughly with 70% ethanol after each condition in order to remove all pheromones. Several controls were used for this experiment to help ensure accurate results and decrease area. The temperature the crickets were held and the time that the males and females were left in the arena were kept constant. The size of the crickets used in each paired fight was kept within 0.1 centimeters in order to prevent an unfair advantage or disadvantage that could alter the results. Using the same female crickets for each condition of each trial also controlled the experiment.

Results

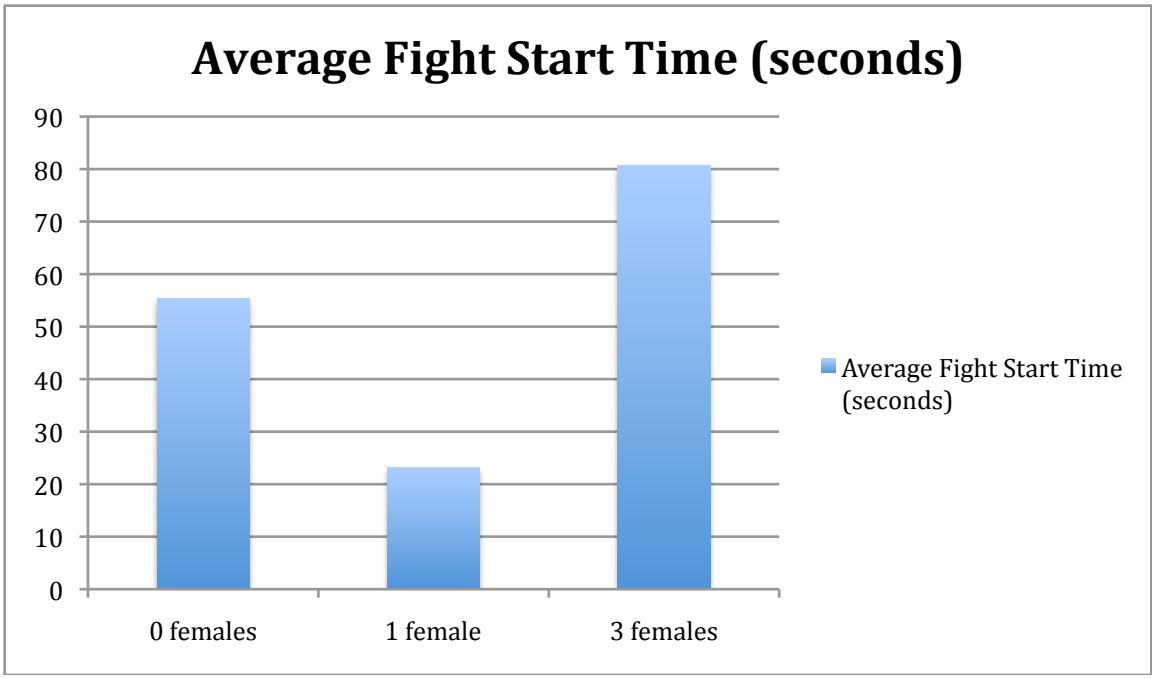
The graph below depicts the differences in the average maximum fight level reached for each condition. It is clear that when exposed to an arena containing pheromones from one female, the male crickets reached the highest average maximum fight level of 4.25 than under the two other conditions. The condition in which the arena contained pheromones from three different females shows the least average maximum fight level of 2.875 reached by the two males. The condition in which no pheromones were present in the arena showed an average maximum fight level of 3.375.



The table below shows the fight start times of each trial under all three conditions. The graph below shows the average time in seconds that it took the male crickets to exhibit aggressive behavior under each condition. The average fight start time was the

quickest for the arena containing pheromones from one female, at 23.25 seconds. The longest average fight start time was for the arena containing pheromones for three females, at 80.75 seconds, and the arena containing zero females had an average fight start time of 55.375 seconds.

	Fight Start Time (seconds)		
Trial Number	0 Females	1 Female	3 Females
1	39	20	18
2	180	7	32
3	66	30	180
4	21	11	6
5	23	8	12
6	56	70	96
7	10	10	122
8	48	30	180



Paired t-tests were conducted between each of the three conditions based on the average fight start time to determine if there was a significant difference between the groups. The t-test between the conditions of pheromones from one female present in the arena compared to three females gave a p-value of 0.024. Since this p-value is less than 0.05, we are able to confirm that there is less than a five percent chance that these two groups come from the same population. This means that we can reject the null hypothesis that there is no significant difference between the average fight start times of the two conditions. The t-test between the conditions of having zero female pheromones present compared to pheromones of one female present gave a p-value of 0.083, and the t-test between the conditions of having zero female pheromones present compared to pheromones of three females present gave a p-value of 0.24. Both of these p-values are less than 0.05, so we were unable to reject the null hypothesis.

The results obtained from this experiment indicate that when exposed to a fighting arena in which pheromones from one female were present, the two male crickets showed more aggressive behavior than when put in the arena that contained pheromones from multiple female pheromones or no female pheromones. This can be supported by the average maximum fighting level reached and the average fight start time displayed in the graphs for the condition in which pheromones for one female were present. The results from the t-test also support this because the conditions in which pheromones were present from one female compared to three females proved to be statistically significant.

Discussion

The results obtained support the hypothesis that when male *Acheta domesticus* crickets were exposed to an area containing pheromones from one female cricket, they would exhibit significantly more aggressive behavior due to mating competition than when exposed to an area with no pheromones or with pheromones from more than one female cricket. The male crickets on average engaged in aggressive behavior more quickly when in a fighting arena that had previously been occupied by one female cricket. This can be explained by the idea that the male crickets sensed the pheromones present from the one female and became more aggressive due to an increase in competition for mating with that female. When exposed to an arena that three females had previously occupied, the male crickets on average took longer to instigate a fight. This supports the idea that the male crickets became less aggressive due to a decrease in competition for female attention since there was evidence of multiple opportunities for mating due to the different pheromones present. According to Arnott and Elwood, the

decision to fight made by animals can be attributed to the assessment of the costs and benefits of competing for resources [1]. This idea could account for the lack of aggression when the male crickets sensed the presence of more than one female. Since the male crickets assessed that mate resource was not scarce, they would not be as motivated to fight for dominance.

These findings can be supported by other research, including a study by Hans Hofmann and Klaus Schildberger that found the most important resources among male crickets to be territories and females [4]. Even though a fight between male crickets constitutes a very small amount of the average daily energy budget, some male cricket species may fight multiple times in order to establish control of a resource, including female crickets [3]. Ogawa and Sakai found that when two male crickets engaged in fighting, the dominant male would express a calling song due to contact chemicals on the body surface [9]. This finding supports our results by relating male cricket aggressiveness to sexual behavior.

Although the results obtained supported the hypothesis, there are many factors that could have caused error in the experiment. Research has shown that contest outcomes between male crickets can be influenced when the crickets engage in multiple consecutive fights. The previous behavioral experiences encountered by crickets in a competition can influence their tendency to fight or avoid aggression [14]. Losing can cause a decrease in willingness to fight again, and winning may increase aggression for the next fight [6]. Since we employed a repeated measures design in which the crickets fought consecutively under three different conditions, the results could have been inaccurate due to the effects of winning or losing the previous fight. Other possible

sources of error were evident in the experiment, including the various conditions of the crickets during the duration of the experiment. In some trials, one of the male crickets had an impaired antenna. In a study conducted by Murakami and Itoh, they found that the removal of both antennae from male crickets limited their male-male aggression [8]. These disabilities might have skewed the results by limiting the cricket's ability to express aggression or providing an unfair advantage for the uninjured cricket. Researcher bias also presents a risk for inaccurate results because the experiment was not blind; therefore, our observational data might have been biased since we knew the conditions for each group. The experiment might have better been controlled by incorporating more strict guidelines and procedures in instigating aggression in the crickets before placing them in the arena, other than the method of tossing them in the air and shaking them in our hands. We could have also controlled the experiment better by providing a more natural environment for the crickets, such as grass or sand in the arena.

From this experiment I gained more understanding of the aggressive behavior of male crickets in relation to the presence of female crickets. From the results obtained, it is clearly evident that the male cricket *Acheta domesticus* exhibits aggressive behavior when threatened with competition for a female mate. This supports previous theories of the explanations behind cricket aggressiveness that can be generalized for providing further knowledge and research on animal aggression.