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Effects of Food and Female Stimulation of Aggression in *Acheta domesticus*

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Abstract: In this study, an experiment was conducted to study the food and sexual factors stimulating aggression. In particular, these were the presence of food and the presence of a female in a transparent container. These factors were studied to analyze if they provided the crickets with a motivation to initiate aggression. The behaviors analyzed were the initial aggression time, the total fight time, the hits per total fight time ratio and the dependence of weight on the fight outcome. The crickets were placed in an arena, acclimated and were allowed to interact afterwards. Two sets of trials were done with the addition of the food stimulus or the addition of the female stimulus. The results demonstrated that there was no statistical significance of the factors in the aggression of the male crickets. It can be concluded from these results that the *Acheta domesticus* may be less inclined to aggression due to the fact that they are not an extremely isolated cricket species which does not motivate a great deal of aggression.

Key words: *Acheta domesticus*, crickets, aggression, female stimulus and food stimulus, trails

INTRODUCTION

Cricket aggression has been studied a great deal by modern behavioral biologists. Aggression has been extensively studied in several isolated cricket species. *Acheta domesticus* also known as the common house cricket, is not an isolated cricket species (Wilson *et al.*, 2010). The males of this species are quite used to being near other males and are not as prone to aggressive behavior as other species of crickets (Hack, 1997a, b). However, when put into an isolated setting, these crickets eventually do participate in combat (Hack, 1997a). Cricket aggression has been found to be induced by octopamine which is a biogenic amine homologous to norepinephrine (Stevenson *et al.*, 2005). It acts as norepinephrine in insects, used for energy-depleting activities which cause stress (Stevenson *et al.*, 2000). The octopamine prevents the production of a particular enzyme that decreases aggression in these insects. Male crickets are found to have an abundance of this neurotransmitter and is often found in larger quantities in species of crickets which have been isolated for a great deal of time (Stevenson *et al.*, 2000; Rillich *et al.*, 2009).

A variety of factors have been known to stimulate aggression in male crickets. In several species of crickets, males are known to fight each other aggressively over females. In one study, the reverse was found to be true the females actually fought over a male (Rillich *et al.*, 2009). Additionally, crickets may exhibit agonistic behavior when placed in an isolated setting for an adequate amount of time with a very limited quantity of food (Hofmann and Schildberger, 2001). The winners are usually those crickets which are most experienced and exhibit a greater size advantage over the other (Dixon and Cade, 1986). However, other factors come into play such as experience and sexual maturity (Hsu *et al.*, 2006). In some cases, aggression is completely avoided due to the fact that the larger cricket has intimidated the other (Stevenson *et al.*, 2000). An examination of the aggression of these male crickets may provide valuable insight into the evolutionary trends in these non-isolated cricket species.

The primary motivational factor in cricket aggression has been observed to be the potential of mating with female crickets of the same species (Ogawa and Sakai, 2009; Rek, 2012). The presence of the female stimulus has been studied by Killian and Allen (2008). In their study, two crickets who had lost earlier fights were pitted against each other with no flight stimulus which reinvigorates the crickets. The presence of a female cricket caused the aggressive behavior to return to the crickets rapidly. The competition among males in other species has also been studied such as in *Gryllus bimaculatus* which is a more isolated type of field cricket (Simmons, 1986). In this case, it is clearly observed that the potential to mate with females is a great motivating factor in cricket aggression.

Various experiments have assessed the aggressive behaviors of crickets in different manners. Often, the experiments study the presence or absence of a particular stimulus (Arnott and Elwood, 2009). In this experiment, the stimulation of aggression was tested in the presence or absence of food and additionally the presence and absence of a female. Afterwards, the morphological traits of the combatants were measured to determine the characteristics which allowed the winners to emerge triumphant. Experimentally manipulating the presence of various stimuli allows for the determination of the causes of the male cricket aggression in this particular species. The comparison to a set of controlled interactions allowed for the determination of the effect these factors had on stimulating the aggressive interactions between the two males of this usually non-aggressive species. It is hypothesized that the presence of the stimuli would provide a motivating factor to the crickets, in terms of aggression. The initial aggression time is expected decrease while the total fight time is expected to increase. This is due to the fact that the first aggressor usually sets the tone for the fight (Rillich *et al*., 2009). The crickets would fight for a longer time, knowing that the winner would have the advantage of obtaining the food pellet or making an impression upon the female present (Hack, 1997a, b). A new measure was created which was the hits per total fight time ratio. This was a measure of aggression density which could give more insight into how the aggression is affected by the stimulus.

MATERIALS AND METHODS

Initially, a set of 30 adult male crickets of the species *Acheta domesticus* was obtained. A large cricket arena with dividers was used with a large quantity of sand spread across the bottom surface to simulate natural surroundings. The experimental apparatus was set up as shown in Fig. 1.

The two combatants were placed in two separate sections. It was important to visualize the colors on the crickets' dorsal side to distinguish them. The crickets had been isolated for up to 48 h without food before experimentation. First, the control group was tested. This group of crickets was allowed to interact without any stimulus. Five pairs of crickets were tested in this group. After allowing 1 min for the crickets to acclimate to the environment, the dividers were lifted and the crickets were allowed to interact. A digital stopwatch was utilized to measure both the time of initiation of the fighting and the duration of the fighting, for a maximum of 5 min. The interactions were observed until a winner was established either by chirping, jumping or subjective observation. After the winner was declared or the 5 min elapsed, the crickets were removed from the container, iced and their morphological traits were measured.

For the food group, the same procedure was set up as in the control except that 1 food pellet was placed in a separate section between the two crickets. Once the crickets were acclimated, the food was also exposed to both crickets. The winner of these fights was established once one had taken possession of the food pellet and by subjective observation. The female group was tested in the same manner except with an adult female cricket of the same species placed in a small transparent plastic container. It is noted that the same female cricket was used in all the trials concerning the female stimulus. The same measurements were taken and the winner was established through the same manner as the control trials.

RESULTS AND DISCUSSION

One of the factors studied was the initial aggression onset time of the combat between the two male crickets. Figure 2 displays the results obtained for this part of the experiment. The initial aggression onset time was measured after the acclimatization had occurred until the first interaction which invoked aggression. From the results, it was observed that the control had the lowest average initial aggression onset time, followed by the presence of the female and finally by the introduction of the food pellet.

Table 1 shows the tabulated data for the initial aggression onset time. As the data was not known to be normally distributed t-tests were generally not utilized. The use of a Wilcoxon signed rank test yielded t-values of 2 and 5 when comparing the control with the food stimulus and female stimulus, respectively which were larger than the t-value of 0.6.

Total fight time was yet another measured factor. The total fight time was measured from the initial aggression fight time until the time at which the winner was decided. This was done through subjective observation as to whether the winning cricket had control over the fight or if it exhibited significant amounts of the triumph

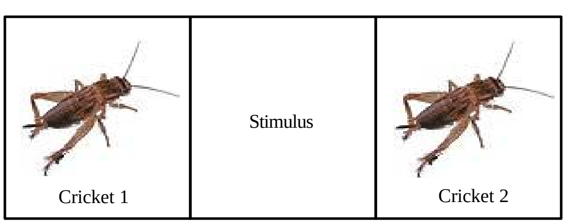

Fig. 1: Experimental apparatus

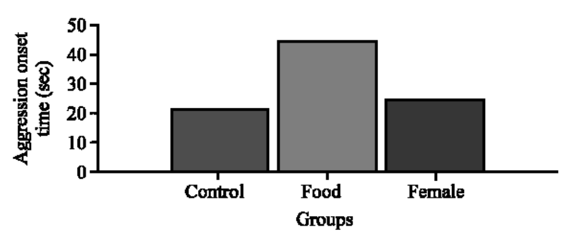

Fig. 2: Initial aggression onset time

Table 1: Initial aggression onset time

Fight No.	Control initial aggression time (sec)	Food initial aggression time (sec)	Female initial aggression time (sec)
1	7.00	42.00	23.00
2	15.00	8.00	31.00
3	22.00	58.00	25.00
4	19.00	68.00	23.00
5	45.00	46.00	21.00
Average (mean)	21.60	44.40	24.60
SD	14.24	22.78	3.85

Table 2: Tabulated total fight time

Fight No.	Control fight time (sec)	Food fight time (sec)	Female fight time (sec)
1	162.00	240.00	190.00
2	157.00	300.00	300.00
3	300.00	300.00	300.00
4	300.00	300.00	300.00
5	190.00	300.00	300.00
Average (mean)	221.80	288.00	278.00
SD	72.49	26.83	49.19

Table 3: Aggression ratio tabular data

Fight No.	Control ratio	Food ratio	Female ratio
1	0.037037037	0.037037	1
2	0.050955414	0.025478	0.5
3	0.056666667	0.018889	0.333333
4	0.016666667	0.004167	0.25
5	0.036842105	0.007368	0.20
Average (mean)	0.04	0.03	0.02
SD	0.02	0.01	0.01

song. Another factor which weighed into the judgment of the winner was jumping. It was observed that the total fight time typically increased with the introduction of a stimulus, compared to the control. The total fight time differences between the control and the stimulated trials typically ranged from 18-25%. The results are displayed in Fig. 3.

Table 2 exhibits the tabulated data for the total fight time. Additional Wilcoxon rank tests were conducted on this data to obtain t-values of 3 for both the food stimulus and female stimulus which was larger than the Wilcoxon table value.

The total number of hits was counted per fight. A hit was defined as an actual physical interaction between the two crickets which was seen as a sign of aggression. This number was then divided by the total cricket fight time. This resulted in a measure of aggression which was actually quantified and could be compared to the subjectively observed aggression rank. The hits per fight time ratio yields a type of aggression density in which the experimenters are able to observe and analyze the effects that external stimuli have on the aggression. From the results in Fig. 4, it was observed that the control group had the highest ratio, followed by the food stimulus group and lastly, by the female stimulus group.

The significance between the control and the stimuli in terms of this ratio was also explored through the Wilcoxon test. The data was tabulated as shown in Table 3. The t-values obtained for the comparison of the

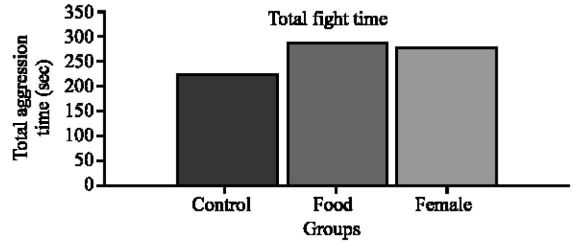

Fig. 3: Total cricket aggression time

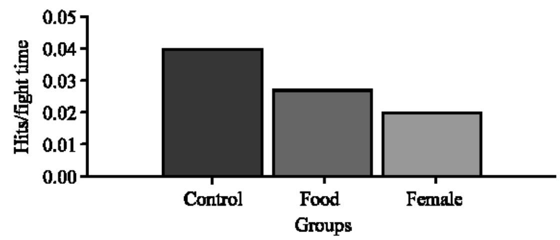

Fig. 4: Hits per fight time ratio

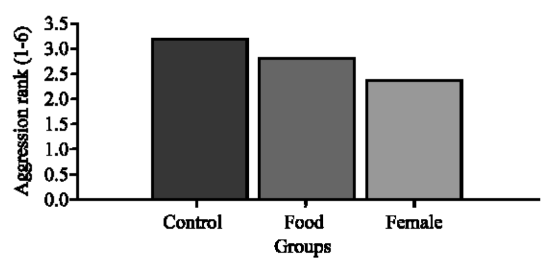

Fig. 5: Average aggression rank

control with the food stimulus and female stimulus, respectively were 15, being larger than the Wilcoxon table value.

The average aggression rank was also graphed to observe if the stimuli provided a greater motivation for the crickets to win the fight. The results are seen in Fig. 5. The control group as in the hits per fight time ratio had the highest average aggression rank. These numbers were obtained by subjective observation and comparison of the observations to the schematic representation of the cricket aggression provided at the laboratory bench. The aggression rank was based on a cartoon schematic where 1-3 were light to moderate interactions and 4-6 were more aggressive actions.

The primary morphological measurement which was analyzed was the weight of the cricket. The weight of the cricket typically gave a good indication as to the relative size of the cricket. The mass dependence of the winner could be observed by graphing the average weight of the winner and the lower for each of the trials. It is observed through the graphical data in Fig. 6 that the winner in the control group and the two stimulus groups was the heavier cricket, on average.

Table 4: Comparison of winner/loser mass

Fight No.	Winner mass (g)	Loser mass (g)
1	0.270	0.300
2	0.230	0.240
3	0.260	0.250
4	0.310	0.270
5	0.370	0.270
6	0.360	0.250
7	0.290	0.230
8	0.270	0.320
9	0.260	0.250
10	0.270	0.190
11	0.190	0.270
12	0.260	0.260
13	0.390	0.230
14	0.350	0.250
15	0.390	0.290
Average	0.298	0.258
SD	0.061	0.031

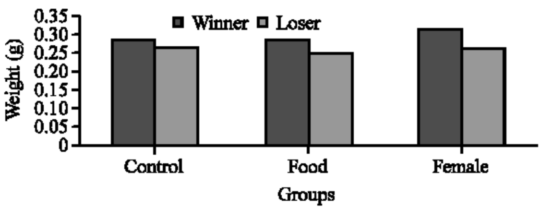

Fig. 6: Fight result mass dependence

A Wilcoxon test was performed to determine the significance of the mass of the cricket to the outcome of the fight. The t-value obtained was 9, larger than the table value. However, if a type III t-test was performed, the p-value was 0.0172 which was lower than the t-table value. The results were tabulated in Table 4.

It was expected that the introduction of either a food or female stimulus would have an effect on the initial aggression time (Wilson *et al*., 2010). It was hypothesized that the presence of a food pellet or a female would actually decrease the initiation time as there would be motivation to win the fight over the other cricket. However, based on the t-tests conducted, a null hypothesis stating that there is no difference between the aggression initiation times between the control group and the stimulus groups could not be rejected. From the results, it was observed that it actually took the crickets, on average, a longer time to initiate aggression when presented with a stimulus when compared to the control. This could be attributed to an increased time in sensing the stimulus which would increase the initiation time as reported by Wilson *et al*. (2010). In terms of overall aggression time, it was expected that the presence of the stimulus would motivate the crickets to fight for a longer period of time (Dixon and Cade, 1986). On average, the results matched the hypothesis. However, from the Wilcoxon tests conducted, there was no statistically significant difference between the stimulus groups and the control.

As was mentioned earlier, a measure of aggression was the hits per fight time ratio. This allowed a measure of the aggression density of the cricket fights. It was expected that this value would increase with the presence of stimuli as there would be a motivation to fight for some kind of stimulus. However, the results did not match expectations. The control, on average had the highest hits per fight time ratio. The Wilcoxon tests, once again, resulted in no statistical significance in terms of the difference of this ratio. Another factor which was expected to be affected was the average aggression rank. The aggression rank was the subjectively observed rank of aggression based on the schematic representation provided. The results did not match the hypothesis that a stimulus would provide the motivation to partake in a more aggressive fight, based on Hoffmann and Shildberger (2001)'s experiment.

For the morphological characteristics which best described the winner, it seemed that the weight of the cricket was the most significant determinant (Wilson *et al*., 2010; Dixon and Cade, 1986). On average, in all three types of trials, the heavier cricket won the duels. Although, the Wilcoxon test demonstrated no statistical significance, the t-test resulted in the rejection of the null hypothesis stating that the any cricket would win the duel regardless of the weight difference. This was a statistically significant result but there were some outliers, where smaller crickets won. This was typically the case in the trials which had the presence of a stimulus which represented a greater driving force for aggression (Wilson *et al*., 2010).

CONCLUSION

Overall, it seemed that the presence of the various stimuli did not affect the aggression of crickets. This indicates an evolutionary significance of the *Acheta domesticus* as they are not an isolated cricket species (Wilson *et al*., 2010). The dense population pattern of this species has allowed it to live cooperatively without increased aggression. Various factors may have contributed to these findings, however. The absence of significance may have been due to the small sample sizes. For each trial, only five pairings were used. Significance may not have resulted in the initial aggression time due to the fact that the crickets may still have been acclimating to their new surroundings. It was observed on several occasions that the crickets were trying to escape the arena. This would have affected the motivation of the crickets to aggressively interact with each other especially if they perceived the new surroundings as an additional threat. The fact that the initial aggression time increased with the presence of the food pellet suggests that the crickets may have been sensing the presence of the food pellet which may have detracted from the onset of aggressive interaction. Additionally, the 5 min maximum given for each fight would have affected the results, especially in the case of the total fight times. The crickets may have continued fighting but due to time constraints, the fights were stopped. Also, the determination of the aggression rank was subjective and in some cases, the experimenters were not unanimous in their judgment. As was earlier mentioned, the species *Acheta domesticus* is typically not inclined to increased aggression due to the fact that in the wild, they are routinely exposed to other males in very close proximity. The species has been known to have a lower abundance of the octopamine neurotransmitter, limiting the aggression (Stevenson *et al*., 2005). This limits the aggression of these types of crickets. This may have affected the data so that significance was not observed even with the presence of the stimuli, regardless of how long the males were kept in isolation.

Another factor which significantly affected the results was the manner in which the winner was judged. This was a very subjective measurement. Initially, it was seen that the winner gave out a sharp trill which signified the triumph song when winning a duel. In the food stimulus group, this was seen as a sign of frustration rather than the triumph song as the cricket without the food usually chirped. Additionally, the time constraints often forced the determination of the winner in evenly matched duels which would have affected the overall fight time results. Another significant observation in the female stimulus group was that the winner usually jumped very high after the duel. This was only observed in this stimulus group, suggesting some kind of courting ritual. A confounding factor in several of the cases was the health of the crickets. In many cases, one cricket would often be missing a body part such as an antenna, a leg or a piece of the mandible, possibly caused by prior fighting. This would have skewed several of the results. These types of crickets would be nearly guaranteed to lose due to loss of sensory mechanisms and fights would often either take place later or the fights would last a very short time (Rillich *et al*., 2007).

Ways to improve this experiment would be to have larger sample sizes. This would make the results more random and accurate and the significance of these results would be found more easily. It is important to note that the standard deviations of the samples were large indicating low precision this would be improved with the larger sample sizes. Additionally, the fighting arena may have been too large to observe the aggression for a large amount of time. It may be better in future experimentation, to test with a smaller arena which would invoke aggressive behavior at a faster rate. Additionally, the determination of the winner should be more consistent. In all trials, they should be determined by one set of criteria, rather than three different criteria which might have affected some results. Also, it would be recommended to carry out the experiment using healthy adult crickets which are on even grounds in terms of having the normal number of body parts. The absence of antennae or legs would often result in the other cricket winning easily.

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