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A Senior Honors Thesis*

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Abstract

Agonistic and aggressive behaviors are a common occurrence within crayfish populations in the natural world. These behaviors appear to be very important to individual crayfish in establishment of their social status and in dominance hierarchies that inevitably form within the populations. An important factor in the founding of these hierarchies and an individual's status within them is the occurrence of winner and loser effects. The winner effect can be defined as the increased probability of an individual winning the next fight after a pervious winning experience. The inverse of this defines the loser effect. The winner effect is a well-established occurrence within crayfish species. However, the loser effect has not yet been as extensively demonstrated. This study was designed to observe the loser effect in a novel crayfish species. The experimental crayfish were first given a losing experience and then the result of their next fight was observed and recorded. The importance of loser effect is in its ability to aid in the formation and stability of dominance hierarchies and also as a contributing factor in the overall fitness of crayfish. The loser effect is beneficial to an individual's fitness, because it leads to behavior that seeks to avoid situations in which the chance for injury is high and increased energy expenditure would occur. Our findings in this study indicate that the loser effect is a statistically reliable event in the novel crayfish species and, further, that it is as strong and stable as the winner effect.

Introduction

Agonistic or aggressive interactions occur in almost all animal species and have been shown to be important in determining an individual's success in accessing resources, which directly impacts their reproductive fitness (Zulandt Schneider et. al. 2001). Agonistic interactions have been studied extensively in many species of crustaceans. The reasons for their use in agonistic behavior research are their unique weaponry (i.e. claws) and use of stereotypical behavior in fights (Bergman et. al. 2003). Common themes of aggression exist among most species of crustaceans. One theme is that many species communicate status information via displays using visual, chemical, or other sensory modalities to create phenomena known as winner and loser effects (Bergman et. al. 2003; Dugatkin & Druen 2004; Rutte et. al. 2006). The winner effect can be defined as the increased probability of one individual winning a fight, following a pervious winning experience. The inverse of this would be defined as the loser effect (Rutte et. al. 2006). And, both effects can be reinforced using aggressive behavior and communication of social status.

These agonistic interactions and their subsequent effects have proven to be important determinates of dominance hierarchies. As individuals within a population interact, they begin to establish social status within a social dominance hierarchy. Once social status is established, individuals then appear to base their subsequent interactions on their given social levels within the hierarchy (Rutte et. al. 2006). For instance, it has been shown that dominant individuals have an increased likelihood of initiating and escalating an agonistic interaction (Goessmann et. al. 2000; Bergman et. al. 2003). Such an establishment of a dominance hierarchy has been theorized to increase the fitness of the dominant individuals (Bergman & Moore 2003) and evidence has also been presented that the hierarchy helps to increase the fitness of the subordinate individuals as well. Subordinates can assess the probability that they will lose fights and simply avoid fights where they would lose and may incur injuries and to

conserve energy (Bergman & Moore 2003; Rutte et. al. 2006). Both winner and loser effects also decrease the length of fights, which has the added benefit of reducing predation risks by minimizing the time spent out in the open when engaged in and agonistic bout.

Although numerous studies have discussed the theoretical presence of the winner and loser effects, many analytical studies have focused solely on the winner effect (Bergman et. al. 2003; Dugatkin & Earley 2004). The loser effect seems to be a rational antithesis of the winner effect, yet is under studied. The central focus of our study was to specifically examine the loser effect. This was accomplished by first giving the experimental crayfish losing or winning experiences by matching the crayfish with another individual that has a significant size advantage or disadvantage. The losing individual then interacted with an individual who was comparable in regards to intrinsic factors (i.e. body size, claw morphology, and size). Matching intrinsic characteristics of crayfish should allow for extrinsic factors, like the loser effect, to be the focus of the experiment.

The importance of the loser effect lies in its ability to add to and determine the structure of the dominance hierarchies. It has been shown in a previous study that the aggressive state of an individual is directly related to previous agonistic success, and it is the aggressive state of the individuals that determines the stability of the hierarchies (Goessmann et. al. 2000). Furthermore, when winner effects operate, the rank of every individual in a hierarchy can be unambiguously determined and tend to be reinforced. While if the loser effect operates alone, only an alpha individual is certain in a hierarchy, while the remaining relationships remain are uncertain and in flux (Dugatkin, 1997). With the winner effect being firmly established (Bergman et. al. 2003; Dugatkin & Druen 2004; Rutte et. al. 2006), we have attempted to demonstrate that the loser effect is both an observable phenomenon and will be used to reinforce social status. We hypothesize that the loser effect will prove to be just as strong and reliable as the winner effect.

Methods and Materials

Animals

For this study crayfish *Orconectes virilis* were captured from area tributaries of Grand River watershed (Fig. 1). Most of the crayfish were obtained from the Rio Grande Creek, near Ravenna Township. Once crayfish were collected, they were tactilely, socially, and chemically isolated in individual ventilated plastic containers for a minimum of one week in the laboratory. This was done to ensure that the social status of the individual was reset, since prior experience influences fights and will continue to influence for up to one week (Bergman et. al. 2003). Each crayfish was fed three times per week with one rabbit food pellet each feeding. The water used in the isolation tanks and experimental tanks was de-chlorinated and maintained at a constant temperature (23˚C). The crayfish were also maintained on a 14-hour light and 10-hour dark cycle. During the experimental fight trials, all crayfish were marked on their dorsal surfaces with white paint to help recognize crayfish for later analysis. Only males were used in this study due to their high baseline aggression levels. At the conclusion of the experiment the crayfish were kept in the lab and later returned to the Grand River watershed.

Experimental Setup

A fight arena was constructed out of black, opaque Plexiglas (51 x 51 x 12.7 cm; Fig. 2). It was divided into four, equal quadrants using retractable walls made of the same Plexiglas. The entire bottom of the arena was covered with white pebbles; these both increased the contrast for viewing the fight and insured a good environment for leverage during the fight trials. The arena was filled with approximately 15 liters of de-chlorinated water that was at room temperature.

Fight Trials for the Loser Effect

In all experimental trials, three crayfish were used per trial. Each crayfish was placed into a separate compartment in the fight arena. After an acclimation period of 15 minutes, crayfish A and B were allowed to interact by retracting wall I (Fig 2). Crayfish A and B were allowed to interact for a single encounter (i.e. until one crayfish retreated or tail-flipped away as the fight loser). Crayfish A was a minimum of 25% larger in carapace (cephalothorax) length, chelae (claw) length, and body mass than crayfish B to ensure a losing experience would be obtained. At the same time, crayfish C was allowed to explore and empty quadrant by pulling wall II. This allowed crayfish C to experience seeing a wall being removed as B did. After crayfish A and B had undergone their one encounter and crayfish C saw the wall being removed, all the crayfish were forced back into their original compartment using a net, ensuring that all the crayfish had similar handling experiences.

After a resting period of 20 minutes after the losing experience trial, crayfish B and C were then allowed to interact by pulling wall III. Crayfish B and C were size-matched crayfish within 10% of carapace length, chelae length, and body mass (see Bergman et. al. 2003 for more details). This was to ensure that size was not the determining factor in the fight, but instead prior fight experience would be the influencing factor. These crayfish were allowed to interact for a single fight encounter (i.e. until one crayfish retreated or tail-flipped away) before concluding the trial. After each trial, the fight tank was cleaned with de-chlorinated water and then refilled with new de-chlorinated water for the next trial to ensure all odors were removed from the tank. During the experiment, 23 trials were performed, using a total of 69 different crayfish. Out of the 23 experimental trials, there were 17 trials where the crayfish that was predicted to lose actually lost. These 17 trials were used in the analysis of the data.

Fight analysis and evaluation

For each experimental trial, a video camera was positioned above the arena and the fight was recorded. The fights were recorded on the digital hard drive on the video camera and then transferred to a computer, for analysis. Each trial was allowed to run for a maximum of 15 minutes per encounter, but was stopped if there was a clear winner in the encounter and one crayfish was in potential danger of injury. The trials were then analyzed using the video and an ethogram adopted from Bergman et. al. 2003 (Table 1). In analyzing each fight trial, the identity of the winner and loser was recorded. The loser was determined when it tail-flipped away from its opponent. In fight trails where the outcome was not as clear-cut, we used the ethogram to ascertain the winner and loser. Each crayfish was assigned a "score" based on fight initiation and the subsequent intensity levels of the fight. By, analyzing the entire fight, we were able to determine a winner and loser. Once the winners and losers for all trials were found, the data was then analyzed using a Tukey multiple comparisons for proportions contingency table ($q_{0.05, \infty, 3} = 3.314$). Results were considered significant with an outcome of $q_{0.05, \infty, 3} > 3.314$, from the multiple comparisons test (Zar, 1999). For this experiment, losing was compared against random behavior, which would be expected to be 50% chance of either winning or losing.

Results

In this study, we conducted 23 total fight trials. Out of these 23 trails, there were 17 trials where the crayfish designated as the "loser," or crayfish B, actually had a losing experience in its first fight. The proportion of second fights lost by a crayfish (B) with a pervious losing experience, were analyzed using a multiple comparisons for proportions contingency table, and comparing the losing proportions against random chance (50-50). Out of the 17 trials used, the crayfish with the previous losing experience lost 13 of the fights and won only 4 of the fights, against a size matched opponent (Fig. 3).

This proportion of fights lost by the "loser" was significantly different than what was predicted for random (q = 4.391); (Fig. 4). By allowing only 15 minutes between the fights in each experimental trial, we ensured that time was not an issue, as a pervious study showed that within a 20 minute interval the crayfish was still influenced by its previous interaction (Bergman et. al. 2003).

Discussion

Our results clearly display that there is an observable loser effect in the agonistic interactions of male *Orconectes virilis* crayfish. Until now, this crayfish species had not had a documented loser effect. Once a crayfish has had a losing experience in an interaction, there was a significantly increased chance of that individual losing in its next interaction. These findings are true in instances where the time between fights is 15 minutes and the second interaction is against a size matched individual. Such a result speaks to the strength of the effect, since only one fight can alter subsequent behavior and fight decisions. We did not test the specific duration of the effect or the specific behavior during the fight, yet our findings indicate that the loser effect is an observable phenomenon within the defined parameters. As demonstrated by earlier studies, the loser effect acts in conjunction with an opposing winner effect, where the individual who had a winning experience had a marked increase in the chance that they would win their next fight (Bergman et. al. 2003; Dugatkin & Druen, 2004; Rutte et. al. 2006).

Interestingly, in our study, we found that the loser effect was demonstrated in individuals who had only a single agonistic encounter. Meaning that after only one experience of losing, the losing individual had a greater chance of losing in its next interaction. We were able to display this in our study, by allowing our experimental crayfish to interact with a substantially larger crayfish until a subordinate relationship was developed. As indicated by previous studies, the winner effect is likely influenced by the up

regulation and release of biological chemicals from the winning individual (Bergman et. al. 2003). These chemicals can lead to changes in neurochemistry and cause the behavior of the winning individual to be altered. Also, these chemicals appear to take part in the communication with other individuals, essentially letting other crayfish opponents that it has a winning fight history. These effects likely lend a hand in the reinforcement of the winner effect. However, as time increases between fights, the neurochemistry returns to normal and the winner effect decreases (Bergman et. al. 2003). Because, there is a change in the neurochemistry of the winning individual it may be likely that there is also a change in the neurochemistry of the losing individual that may help establish the loser effect. Again, our study did not focus on this aspect of the loser effect, but this is one aspect of the loser effect that is open for continued studies.

The results of our experiment indicate the possible role that winner and loser effects have on the foundation of dominance hierarchies. Throughout the natural world, there are many instances of the dominance hierarchies that are established among populations of organisms. These hierarchies are developed through a series of agonistic interactions, where individuals either emerge as dominants, subordinates, or even intermediaries. As these interactions progress in populations, individuals appear to be able to recognize which individuals are more dominant and which ones are not; eventually leading to the establishment of dominance hierarchies, which are based on individual or status recognition (Goessmann et. al. 1999; Dugatkin & Druen, 2004). In crayfish, it has been shown that in every instance where crayfish are allowed to interact repeatedly, a linear hierarchy will be established (Goessmann et. al 2000). Clearly, this demonstrates that dominance hierarchies are a very important aspect of the social life of crayfish.

With the confirmation of the existence of the loser effect in crayfish, we can now couple it with the many findings giving evidence for the winner effect (Bergman et. al. 2003; Dugatkin & Earley 2004), to

see that these two opposite effects seem to work together in establishing relationships among crayfish and help in the establishment of dominance hierarchies. It has been displayed that the chemical changes occurring in crayfish after a winning experience may help other crayfish recognize them as a winner (Bergman et. al. 2003). If a similar phenomenon occurs with the loser effect, then the winner and loser effect would both work in a population to help the individuals recognize each other as either winners or losers. Status recognition has already been shown to occur within crayfish populations in previous studies, where crayfish were observed to alter their behavior based on the status of their opponent (Zulandt Schneider et. al. 2001). Further, it has been shown that an individual's status and behavior is dependent on their previous agonistic success, indicating that their status is dependent on either the winner or loser effect (Dugatkin, 1997).

In our study, we were able to clearly display the existence of the loser effect in crayfish. It appears that the winner and loser effect are both very important in the establishment of dominance hierarchies in crayfish, but why do winner and loser effects even exist? It is evident from previous studies that the winner effect is likely the result of changes in the neurochemistry of the individuals (Bergman et. al. 2003), but what also has been shown is that there may be very important extrinsic factors which may lead to the existence of a winner effect. One study found that things like the availability of shelter and food, have a marked effect on the behavior of an individual, causing crayfish to act more aggressively in response to these resources (Bergman & Moore, 2003). Clearly, the winner effect would help an individual gain more resources if it had to fight to gain them, simply because they have a higher probability of winning after a pervious win. But, what advantage would the loser effect entail? This same study showed that an individual can increase its own fitness in other ways than winning fights to gain resources. The fitness of the individual would certainly be increased if they were to avoid fights in which they had a large risk of being seriously injured or expending a lot of energy (Bergman & Moore, 2003). In this way they loser effect would be very important, if an individual could assess its own status

based on losing or winning pervious fights, then they could avoid situation in which the probability of them losing was high. This would certainly increase their fitness and serve to solidify the loser effect as an evolutionarily important occurrence. There remains much opportunity to expand the knowledge of the importance of and the mechanisms behind the existence of the loser effect, which can now be explored with the confirmation of an observable loser effect in this novel species.

Figures

Figure 1: *Orconectes virilis* crayfish collected from the Grand River system.

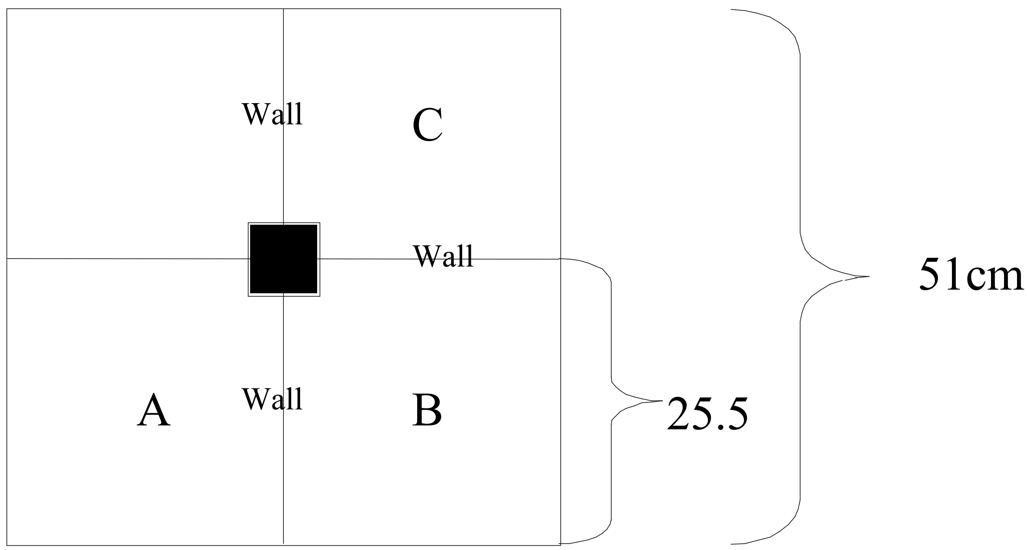

Figure 2: Initial fight trial set up. This figure displays the fight arena that will be used in the experimental trials.

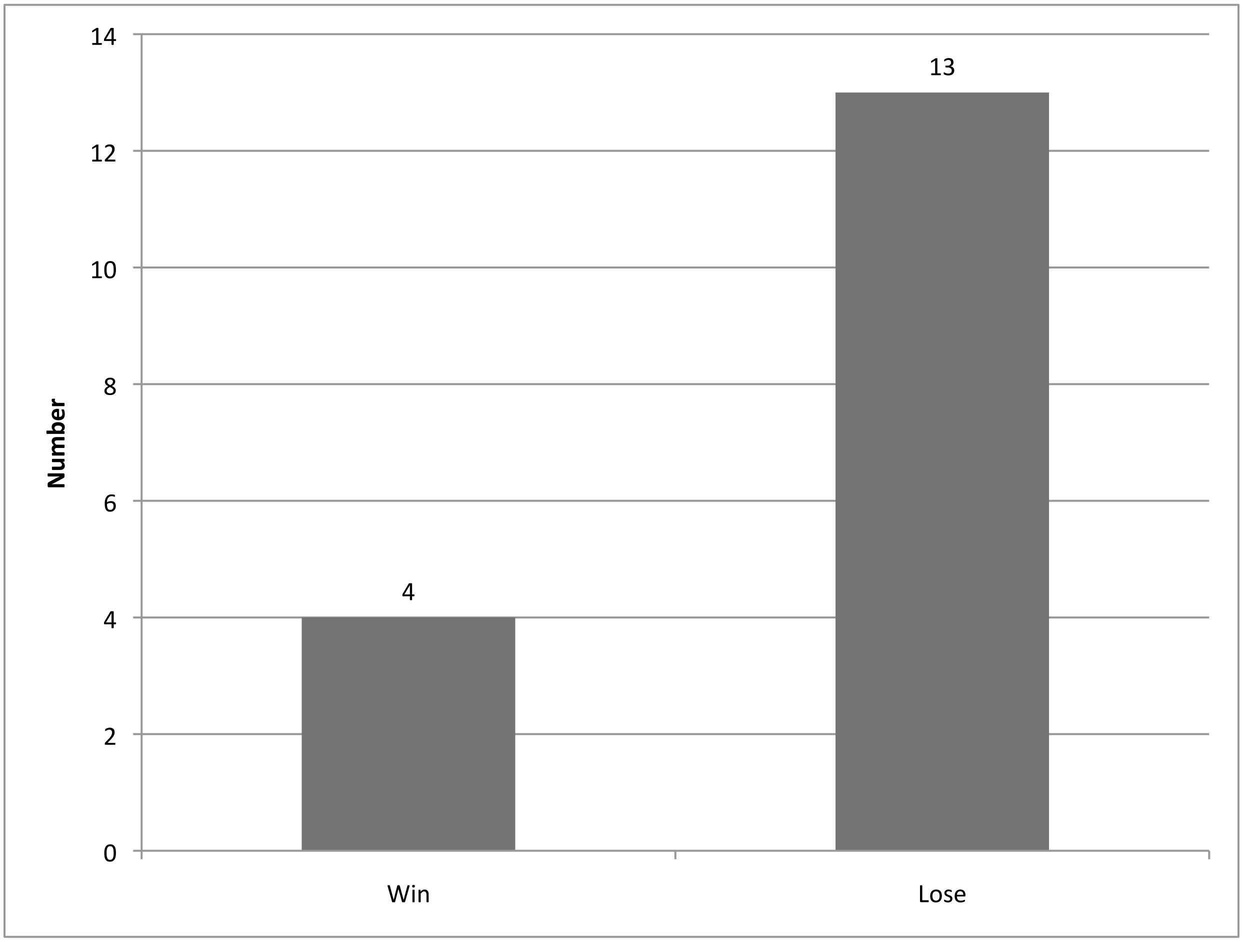

Figure 3. The number of fights that the "loser" crayfish (B) won and lost in all the experimental fight trials. (N=17)

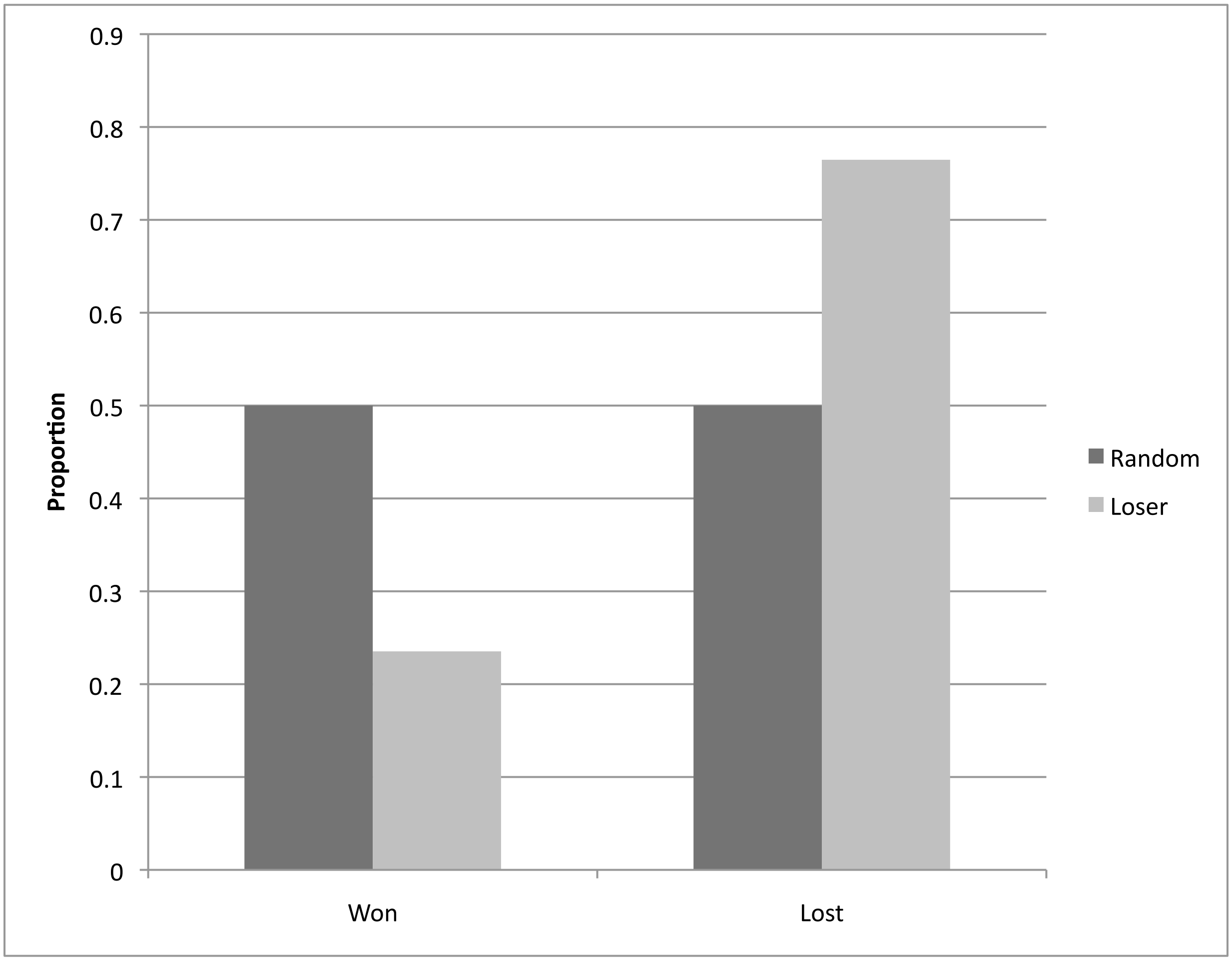

Figure 4. Proportion of fight won and lost by the crayfish with a pervious losing experience (N=17 for the experiment). There is a significant difference between the outcomes for the "loser" crayfish and random for losing in the next encounter ($q_{0.05, \infty, 3} > 3.314$).

Table 1. Crayfish ethogram codes, used to determine winner and loser in trials.

Intensity Level	Description
−2	Tailflip away from opponent or fast retreat
−1	Retreat by slowly backing away from opponent
0	Visually ignore opponent with no response or threat display
1	Approach without a threat display
2	Approach with meral spread threat display usually accompanied by an antennal whip
3	Initial claw use by boxing, pushing and/or touching with closed claws
4	Active claw use by grabbing and/or holding opponent
5	Unrestrained fighting by pulling at opponent's claws or body parts

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