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Winner and Loser Effects in Madagascar Hissing Cockroaches (Gromphadorhina portentosa)

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WINNER AND LOSER EFFECTS IN MADAGASCAR HISSING COCKROACHES

(*Gromphadorhina portentosa)*

by

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Abstract

Winner and loser effects have been documented in many species throughout the animal kingdom, but have yet to be investigated in Madagascar hissing cockroaches. This study seeks to determine if winner or loser effects are present in Madagascar hissing cockroaches. In Experiment 1, three subjects won a training contest and three subjects lost a training contest. Training contest winner and training contest loser subjects were then paired up in test contests. Four of the 6 subjects showed test contest outcomes consistent with their training contest outcomes, but the overall conclusions were ambiguous due to the small sample size. Whether the results supported the social-cue or self-assessment hypothesis could not be determined because both subjects had a previous social experience. In Experiment 2, four subjects won their training contests and six subjects lost their training contests before being paired in a test contest against a neutral, inexperienced rival. Eight of 10 subjects showed test contest outcomes consistent with their training contest outcomes, suggesting both winner and loser effects. The results of Experiment 2 additionally provide support for the self-assessment hypothesis of winner and loser effects in this species, which suggests that individuals reevaluate their own abilities based on their outcomes from previous interactions.

Introduction

Aggressive and submissive behaviors are integral to defining the outcomes of contests between rivals as well as to the formation of dominance hierarchies. These hierarchies are often a central organizing feature of animal groups, dictating access to resources such as food, mates, territory, and social support (Lindquist & Chase, 2009). The likelihood that an animal will produce an aggressive or submissive behavior is influenced both by personal factors, such as the subject's size, age or weight (Chase et al., 1994), and experiential factors, such as social support or history of winning or losing a contest (Dugatkin & Earley, 2004; Abe et al., 2020). For example, there is an increased chance of winning an aggressive interaction after having previously won an aggressive interaction (Jackson, 1991, Hsu et al., 2009, Rutte et al., 2006) and an increased chance of losing after having previously lost (Hsu et al., 2006, Huhman et al., 2003). These are known as the winner and loser effects respectively, and have been found in many different species ranging from human males (Page & Coates, 2017) to juvenile crickets (Abe et al. 2020).

Winner and loser effects

Winner effects increase the subjects' drive to escalate contests (Hsu et al., 2006, Otronen, 1990). The winner effect has been shown across many species to make an individual more aggressive and sometimes twice as likely to win contests following a win (see Hsu et al., 2006, Rutte et al., 2006 for reviews). The winner effect may primarily be driven by a combination of two factors. First, the animal who initiates an aggressive interaction usually wins that interaction (Hsu et al., 2009), and second, winners of previous contests are more likely to initiate future aggressive interactions (Jackson, 1991). Both the physical exertion of energy to perform aggressive actions, as well as the rewarding aspects of a win, increase the inclination to engage in a future fight (Stevenson & Rillich, 2012). The winner effect can increase the resource holding power of an individual by increasing their access to resources through contest wins (Hsu et al. 2006).

In contrast, loser effects have been reported to dissuade subjects from fully engaging in contests (Hsu et al., 2006, Otronen, 1990). In field crickets, consistently losing causes individuals to surrender early in their next competition (Alexander, 1961). In some species, like pumpkinseed sunfish (*Lepomis gibbosus)*, an individual who has recently lost an interaction will almost always lose a subsequent interaction against a neutral animal (Beacham & Newman, 1987). Large pumpkinseed fish who lose a contest are even more likely to lose a subsequent contest to a smaller individual (Beacham, 1988), highlighting the strength of the loser effect. Indeed, in a synthesis of 14 studies of fish, reptile, and mollusk species, the chances of losing a contest are 5x more for a subject that has previously lost (Rutte et al., 2006). In most species that exhibit both winner and loser effects, the loser effects were stronger in terms of retention and significance (Chase et al., 1994, Rutte et al., 2006).

Both winner and loser effects, especially in invertebrates, vary in duration and influence across species (Chase et al., 1994). In juvenile crickets (*Gryllus bimaculatus)*, loser effects lasted more than two hours, while winner effects were retained for less than two hours (Abe et al., 2020). Lobster cockroaches, on the other hand, show winner and loser effects that disappear and reappear, thus leading their social hierarchy to be ever-changing (Kou et al., 2019). Winner and loser effects were found in jumping spiders to have similar magnitudes, but retention of the winner effect did not last as long as the loser effect (Kasumovic et al., 2010). This variability indicates that winner and loser effects are not fixed across invertebrates, they vary in length and strength.

How do winner and loser effects work?

Because aggressive interactions are costly, using previous contest outcomes to dictate how aggressively or submissively to behave on the next social encounter would allow individuals to avoid a potentially costly losing battle (Clarke & Moore, 1994). Winner and loser effects benefit the individual by reducing the need for physical contests in aggressive interactions (Whitehouse, 1997) and assisting individuals in making the quick 'fight or flight' judgments prior to an aggressive interaction. Two theories have been proposed to explain the mechanism behind winner and loser effects: the self-assessment hypothesis and the social-cue hypothesis. Importantly, these hypotheses are supported by research, but they are not mutually exclusive, indicating that an individual or species might employ either or both the self-assessment and the social cue hypothesis.

The self-assessment hypothesis suggests that individuals re-estimate their own fighting abilities based on the outcome of their previous aggressive interactions (Hsu et al., 2009, Whitehouse, 1997). Thus, an individual's confidence in itself affects its expected costs of engaging in a contest, and influences decisions and probability of winning a fight (Hsu et al., 2009). Winning a contest would raise an individual's estimation of its own fighting ability, increasing their interest in engaging in future contests and increasing their chances of winning subsequent contests. In contrast, losing a contest would lower that estimation, increasing reluctance to engage in another contest and decreasing the odds of winning (Hsu et al., 2006, Huhman et al., 2003).

The social-cue hypothesis suggests that animals read cues produced by opponents to evaluate their own fighting abilities in comparison to the opponent's (Rutte et al., 2006, Hsu et al., 2009). These cues include behavioral displays of aggression, exhaustion, or injury, as well as chemical signals. In this case, an animal who recently won a contest would project aggressive signals to its subsequent rivals. These rivals might then assume that the animal would defeat them in a contest, and show submissive behavior to limit the cost of the fight instead of reciprocating the aggression. On the other hand, an animal who recently lost a contest would project submissive signals to its subsequent contest partners, leading these rivals to assume the animal would lose the contest, and causing them to show increased aggression. In support of this hypothesis, it is well established that cues about other individuals are taken into account in social interactions across species. A sparrow will assess its own fighting abilities by gathering information from how another individual reacts to it (like avoiding it or displaying aggression or submission towards it; Rohwer, 1985).

Madagascar Hissing Cockroaches

Male Madagascar hissing cockroaches (*Gromphadorhina portentosa*) have linear dominance hierarchies that determine access to food, territory, and mating partners (Guerra & Mason, 2005, Clark & Moore, 1994). Males of a higher rank have often been observed inhibiting directly through interference, or indirectly through their presence, mating attempts of subordinate males (Clark, 1998). Aggression and submission are important in the creation and maintenance of this hierarchy (Clark & Moore, 1995b). Previous research has shown that personal factors, or factors that can be measured before a contest, such as size, age, or weight, do influence contest outcomes in Madagascar hissing cockroaches, such that males that hiss more loudly or males that are larger are more likely to win a contest (Clarke & Moore, 1994). However, these attributes do not account completely for linear dominance structures (Lindquist & Chase, 2009). There has been no published research on whether or how experiential factors like winner and loser effects relate to contest outcomes and hierarchy formation in this species. However, the maintenance of the hierarchy in this species occurs mainly through displays such as hissing, physical attributes like size, and behaviors like aggression (Clark & Moore, 1995b). Display cues do not involve physical interaction with the opponent at all, but can be considered passive wins for the individual they were performed against, who counts them with the interactions they initiated in their self-reflection (Jackson, 1991). When physical attributes are obvious enough, or when aggression is deemed too costly, animals are expected to settle interactions quickly and without escalation (Parker, 1974), which allows an individual to maintain dominance without continuous aggression, saving energy and injury (Clark & Moore, 1995a) for both senders and receivers; but, when they are not unambiguously perceived, a fight for dominance must ensue.

Present study

We determined the extent to which Madagascar hissing cockroaches show winner and loser effects in the present study. Adult male Madagascar hissing cockroaches engaged in fixed training interactions. Subjects were presented with either a highly aggressive stimulus animal with a history of winning, such that the subject was likely to lose the social interaction (conditioned loser subject), or presented with a highly submissive stimulus animal, such that the subject was likely to win the interaction (conditioned winner subject). After a short break, subjects were tested against a new opponent that experienced the opposite training outcome. The outcomes of the test contests were recorded to see if training contest outcome was predictive of test contest outcome (Hsu et al., 2009). If winner and loser effects impact contest outcomes in this species, males that won their training contest will show higher levels of aggression, lower levels of submission, and increased likelihood of winning the test contest than males that lost the training interactions.

Experiment 1 -Winners vs Losers

In Experiment 1, we tested for winner and loser effects in contests with a conditioned winner subject paired against a conditioned loser subject. If winner and loser effects play a role in contest outcomes in this species, we expect to see both winner and loser effects simultaneously, so the outcome of test contests would be quick and clear.

Methods

Subjects & Stimuli

Subjects were six adult male Madagascar hissing cockroaches (*Gromphadorhina portentosa*). All subjects were born in the Madagascar hissing cockroach facility at Bucknell University, and spent their entire lives in our colony. Subjects varied in age between 1-3 years, and had an average weight of 7.81g. Only male cockroaches were used because only males show pairwise aggression and dominance hierarchy formation (Clark & Shanklin, 1995).

Two stimulus animals were used for the training phase. One had a history of winning contests, was highly aggressive, and was larger in mass than the subjects by at least 1g. The other had a history of losing contests and showing submissive behavior, and was smaller than the subjects by at least 1g.

Housing

All roaches were isolated for at least one month prior to the start of the experiment in attempts to eliminate any lingering winner and loser effects from previous social experiences (Kou et al. 2019). Subjects were housed individually in plastic “Kritter Keeper” containers approximately 7.13"L x 4.38" W x 5.5"H, or plastic drawers approximately 13"L x 8.50"W x 5.38"H. Since Madagascar hissing cockroaches are nocturnal, the lab operates on a reverse light-dark cycle, with dark lighting from 8:00 am to 8:30 pm. This experiment ran during the dark cycle under red lighting, which did not disturb the light cycle. Each housing unit contained a one-inch thick layer of Zoo Med Eco Earth loose Coconut Fiber substrate, an egg-crate shelter, and two petri dishes, one for food and one for water crystals. The diet of the roaches consisted of wheat germ and Mizuri primate chow crumbs and, along with water, was available *ad libitum* except during testing and training sessions. The temperature of the housing room was 26℃.

Design

Each subject roach took part in two contests: a training contest against a stimulus roach, and a test contest against another subject roach (Figure 1). All pairs of subjects for the test contests were selected to match body weight as closely as possible. All test pair weight differences were within 5.31% of their body weight. Aggressive acts are more likely to occur in similarly ranked/characterized pairings (Jackson, 1991), so size matching increased the likelihood of aggression occurring during the contest, as well as eliminate any size biases that would favor one animal over the other in contest outcomes. Since the sizes of the subjects varied by 5.31%, the smaller subject was always assigned to the winning training condition to avoid size being an explanation for test fight outcomes, as empirical data shows it has a large influence over contest outcomes (Clark & Moore, 1995a).

For the subjects in each test contest pair, our goal was for one subject to win their training contest and for one subject to lose their training contest. We attempted to fix these training contests by pairing one subject with an aggressive stimulus roach who was larger than all of the subjects and had a history of high levels of aggression and of winning fights. The subject was therefore expected to lose the training contest against this stimulus roach. The other subject was paired with a submissive stimulus roach who was smaller than the subject and had a history of low aggression and of losing fights. The subject was expected to win the training contest against this stimulus roach.

The original design of this experiment was to present each subject with both conditions, pitting them once against a submissive stimulus animal and once against an aggressive stimulus animal, with the two conditions separated by four weeks. However, three of the second contests did not yield clear results due to inactivity or tied ratios, and many of the other subjects that experienced the first condition died in the isolation period before the second condition. We therefore only present data from the first contest for each subject in this experiment.

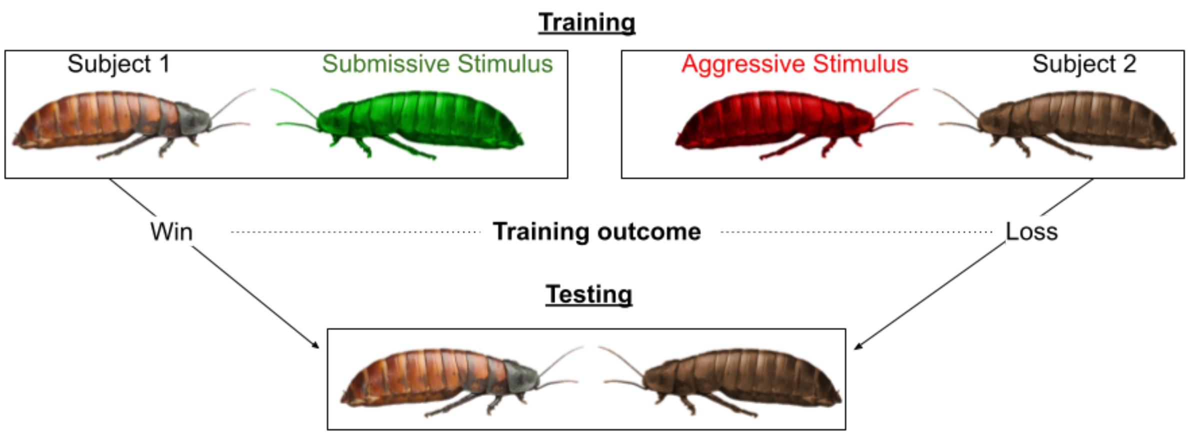

Figure 1: Training and testing schematic for Experiment 1

Procedure

Training. All contests, training and test, took place in an approximately 12 inch rectangular clear acrylic arena. All walls of the arena had a layer of petroleum jelly across the top to prevent subjects from escaping the arena. A movable opaque partition was placed in the center of the arena (Figure 2). The subject roach was placed in the arena on one side of the divider, and the stimulus roach was placed on the other side of the divider. After a five minute acclimation period, the opaque partition separating the two roaches was lifted and the roaches were allowed to interact in the arena for 30 minutes. After this time, roaches were placed back in their home container to rest for one hour before the testing phase. Because of the dramatic differences in size between the subject and the stimulus roach, the contest was expected to resolve quickly.

Training sessions for the two subject roaches in a given test pairing took place simultaneously, in separate arenas (Figure 1). Therefore at the test, both the winning and losing subject roaches had equally recent experience in the testing arena, but differed in the outcome of their most recent contest. All training and test trials were video recorded.

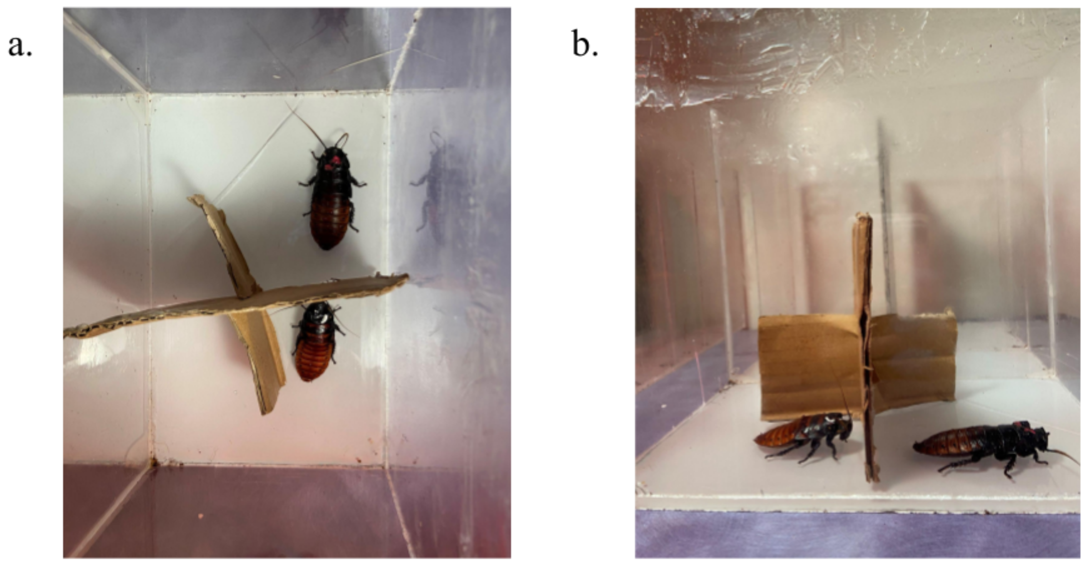

Figure 2: Images of the contest arena (a) top view, and (b) front view

Testing

The testing phase began 60 minutes after the conclusion of the training phase. The pre-selected pairs of subjects, one who had recently won a training contest and one who had recently lost a training contest, were placed in the arena with the opaque divider separating them. After a five-minute acclimation period, the divider was lifted and the roaches were allowed to interact for 30 minutes.

Data Collection & Analysis

During both the training and test contests, the behavior of each individual roach was scored live by an experimenter according to an ethogram (see Appendix 1). The ethogram defines the recorded behaviors of interest for this experiment and was modified from the Madagascar Hissing Cockroach ethogram established by Clark and Moore (1994). All dominance (climb on, head butt, push, attack, wrestle, waggle, hiss, head down, posture, abdominal thrust, approach) and submissive (withdraw, freeze, side tilt) behaviors were recorded and counted, as well as the winner of the contest, and which roach engaged in the first act of aggressive contact (climb on, head butt, push, attack, wrestle). If no interaction was observed for the first 5-10 minutes in the beginning of the session, the opaque divider was reinserted into the arena, with both subjects on the same side, to decrease the available area for the subjects and increase the probability of interaction.

We determine the winners and losers of both the training and test contests using a ratio of submissive behaviors to dominant behaviors. This dominance ratio is displayed as the number of dominant to number of submissive behaviors (dom:sub in Tables 1-4). Included also was the percentage of dominant behaviors out of total behaviors performed by each individual. Comparing these percentages was ultimately how the winners of each contest were determined, and the dominance ratios validated these determinations. Any contest in which there was no clear winner or loser was ruled inconclusive and therefore not included in analyses. An inconclusive contest resulted from no contact being made, or behavior equating to the same ratio in each rival.

R statistical software was used to analyze data for all training and test contests. Behavior during training contests was analyzed using a linear mixed model, with the number of behaviors as the dependent variable, the outcome of the training contest (win or loss) and the behavior type (dominance or submission) as fixed effects, and subject as a random effect. Similarly, behavior during test contests was analyzed using a linear mixed model, with the number of behaviors as the dependent variable, the outcome of the training contest (win or loss) and the behavior type (dominance or submission) as fixed effects, and subject as a random effect. A probability value of $p = 0.05$ was used to test significance.

Results and Discussion

Training

As intended so the testing pairs could be matched within the subject pool, the training sessions yielded three subjects that won their training contests and three that lost (Table 1). In the training contest, the subjects paired against the submissive stimulus animal exhibited minimal submissive behaviors (0, 1, and 2 total behaviors performed by S1, S2, and S6, respectively). All three subjects who experienced a contest against the aggressive stimulus roach exhibited only submissive behaviors, no dominant or aggressive behaviors.

Training Pair	Winner	dom: sub	% dom	Loser	dom:sub	% dom
1	S1	95:0	100%	SB	5:9	36%
2	S2	12:2	86%	SB	2:5	40%
3	S6	3:1	75%	SB	4:3	57%
4	AG	58:1	98%	S3	0:8	0%
5	AG	42:0	100%	S4	0:3	0%
6	AG	48:0	100%	S5	0:3	0%

Table 1. Training contest outcomes for Experiment 1. The ratio of submissive to dominant behaviors was used to determine the winners and losers of the six training contests. S indicates subjects, AG indicates the aggressive stimulus roach, SB indicates the submissive stimulus roach. Roaches with smaller ratios, which indicated fewer submissive behaviors, were scored as contest winners.

There was no main effect of training contest outcome on the number of behaviors shown during training contests ($F_{1, 3.77} = 3.808$, $p = 0.127$). However, there was a significant main effect of behavior type, such that animals showed more dominance behavior than submissive behavior during training ($F_{1, 12.2}$=10.241, $p = 0.0075$). This is to be expected, as this species has more behavioral options that indicate dominance than submission (Appendix 1). Importantly, there was a significant interaction between training contest outcome and behavior type, such that subjects that won their training contests exhibited more dominance behavior than subjects that lost their training contests (Figure 3; $F_{1, 12.2}$= 14.04, $p = 0.0027$). This corroborates our classification of contest “winners” and “losers.”

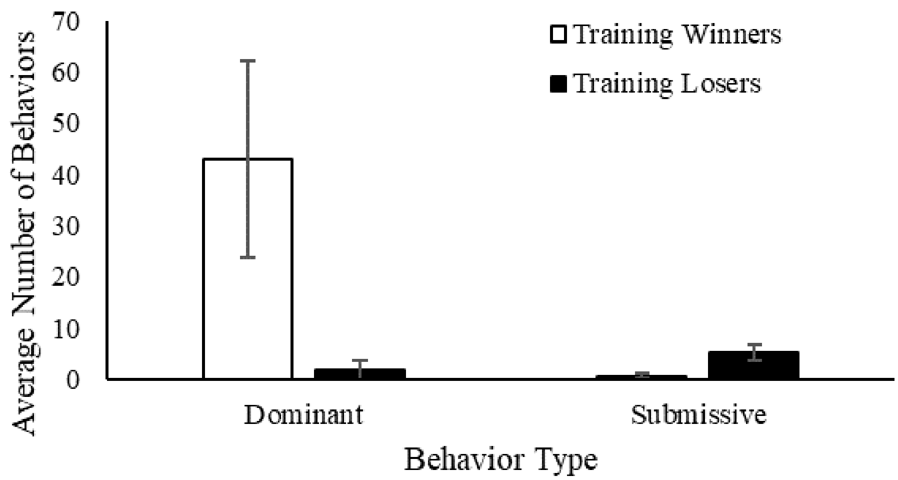

Figure 3: Average number of behaviors during the training contest for winners and losers, broken down by behavior types. Error bars represent standard errors.

Test

Four of the six subjects showed test contest outcomes consistent with their training contest outcomes (Figure 4, Table 2). While this effect did not differ significantly from chance (binomial test, chance=0.5, p=.109), it is difficult to conclude from this small sample size if this is due to the absence of winner and or loser effects, or simply to the small sample. Two of the three subjects that lost their training content made the first contact in their test contests, while only one out of three subjects who won their training contest made the first contact in their test (Figure 4, Table 2).

Test Pair	Winner					Loser				
	ID	Training outcome	dom:sub	% dom	First Contact	ID	Training outcome	dom:sub	% dom	First Contact
1	S1	Win	186:0	100%	No	S3	Loss	17:5	77%	Yes
2	S2	Win	2:1	67%	Yes	S5	Loss	2:3	40%	No
3	S4	Loss	122:0	100%	Yes	S6	Win	1:14	7%	No

Table 2. Outcomes of test contests organized by the winner (left) and loser (right) of each test contest. Training outcome indicates the outcomes of that subject's training contest. The dom:sub ratio indicates the amount of dominant to submissive behavior shown by the subject in the test contest. % dominance indicates how many of the subjects' performed actions were considered dominant. First contact indicates whether the subject made the first aggressive contact in the test contest.

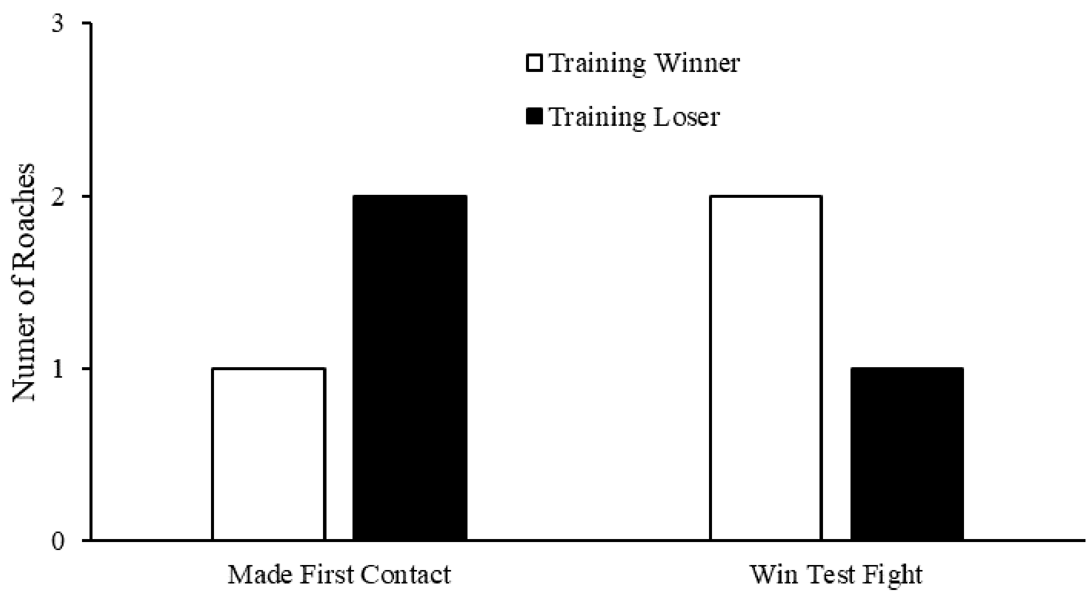

Figure 4. Number of subjects that made first contact (left) and that won their test contest (right) broken down by the subjects that won or lost their training contest.

Neither the training outcome ($F_{1,8}$ = 0.07, p = .793) nor the behavior type ($F_{1,8}$ = 2.06, p = .189) had a measurable effect on the behaviors shown by subjects during the test contest. Further, there was no significant interaction between training outcome and behavior type on the number of behaviors exhibited ($F_{1,8}$ = 0.03, p = 0.869; Figure 5).

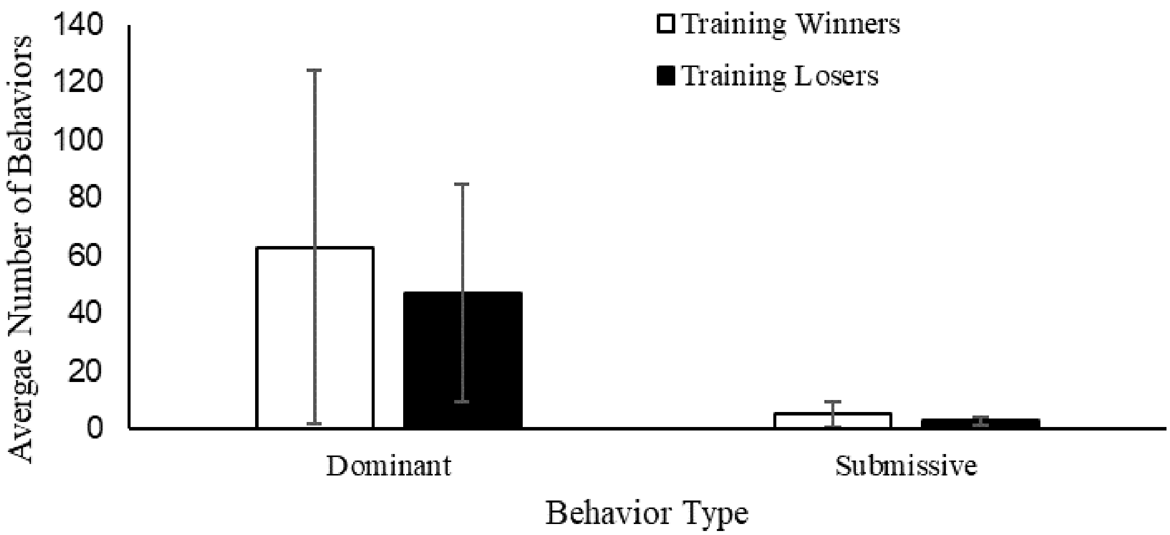

Figure 5: Average number of behaviors in the testing session for winners and losers of the training session. Error bars represent standard error.

The results of this experiment are ultimately inconclusive in determining the roles of winner and loser effects on contest outcomes in Madagascar hissing cockroaches. Consistent with winner loser effects, four of the six subjects showed test contest outcomes consistent with their training contest outcomes. However, inconsistent with the winner and loser effects, training contest winners were not more likely to initiate aggressive contact during test contests, and training contest outcomes did not predict dominance or submissive behaviors during the test contests. A major limitation of this experiment was the small sample size for test contests. In addition, while the experimental design of pairing training contest winners and losers together for test contests was made to maximize the likelihood of discovering winner and loser effects if they were present in this species, this design does not allow us to disentangle the contributions of the social-cue and self-assessment hypotheses to winner and loser effects. In Experiment 2 we expand the sample size and modify the procedures to allow for separation of the winner and loser effects.

Experiment 2: Neutrals

In Experiment 1 we saw trends towards winner and loser effects, such that experience in training contests may have predicted the outcome of test contests. However, because the test contests paired subjects who had won training contests with subjects who had lost training contests, it is not possible to determine if the test contest outcomes were due to winner effects alone, loser effects alone, or a combination of the two. Additionally, the low subject numbers made it difficult to draw conclusions about these effects. Therefore, in Experiment 2, more subjects were used, and the subject roaches were partnered with neutral roaches, who had not experienced any fights within the month prior to the test contest. This eliminates the possibility for the subject to use the social-cue mechanism of evaluation before a contest, because the neutral partner roach will not have had prior contests to build their social-cue repertoire. Further, since each contest was against a naive partner, the only variable between the contests was the individual subject's self-evaluation.

Methods

Subjects & Housing

Subjects were 10 adult male Madagascar hissing cockroaches (average weight: 8.26g) and partner roaches were 10 adult male Madagascar hissing cockroaches (average weight: 8.31g). As in Experiment 1, two stimulus roaches were also used, one aggressive and one submissive. The housing conditions were identical to Experiment 1.

Design

Each subject roach took part in two contests: a training contest against an aggressive or submissive stimulus roach, and a test contest against a neutral partner roach (Figure 2). Neutral partner roaches for the test contest were selected to match the subject's body weight as closely as possible. All test pair weight differences were within ± 7.03% of their body weight.

As in Experiment 1, the goal was for half of the subjects to win their training contest and for half of the subjects to lose their training contest. We attempted to fix these training contests using the same methods as in Experiment 1. Half of the subjects were paired with an aggressive stimulus roach who was larger than the subject and had a history of high levels of aggression and of winning fights. These subjects were therefore expected to lose the training contest against this stimulus roach. The other half of the subjects were paired with a submissive stimulus roach who was smaller than the subject and had a history of low aggression and of losing fights. These subjects were expected to win the training contest against this stimulus roach. Neutral partner roaches did not participate in a training contest. Therefore their first contest was the test contest against a subject roach (Figure 6).

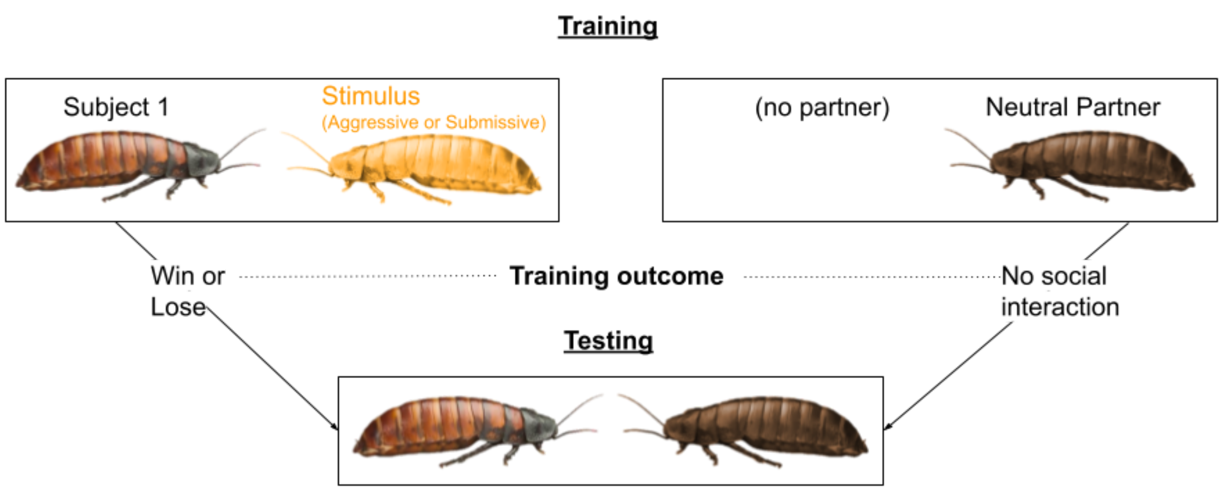

Figure 6: Training and testing schematic for Experiment 2

Procedure

Training

The training contests for the winner and loser trained subject roaches followed the same methods as in Experiment 1 (see Figure 2 for experimental set-up), though had new individuals in the submissive and aggressive stimulus roles (the aggressive stimulus was still at least 1g larger than the subject and had a history of winning, and the submissive stimulus was still at least 1g smaller and had a history of losing). During training, the neutral partner roaches were likewise placed in a contest arena for 30 minutes, but there was no conspecific present (Figure 6).

Test

The procedure was identical to that used in Experiment 1 for the testing contests, except that the subjects were paired with a rival neutral animal that had no previous social experience. Again, if no contact was made within the first 5-10 minutes, the opaque divider was reinserted with both individuals on the same side in order to decrease the amount of space in the testing arena.

Data analysis

Data were recorded and scored using the same methods as in Experiment 1. Additionally, to determine if there was a relationship between the magnitude of the training contest outcome and the magnitude of the test contest outcome, a correlation analysis was conducted on the training and testing ratios for each subject.

Results and Discussion

Training

Of the 10 training contests, four subjects won their training match and six subjects lost their training match (Table 3). Training outcome did not have a significant effect on the number of behaviors subjects produced during the training contest (LMM main effect of training outcome: $F_{1,36} = 3.06$, $p = 0.089$). As in Experiment 1, the roaches showed more dominance than submissive behaviors (LMM main effect of behavior type: $F_{1,36} = 8.26$, $p = .007$). While there was no significant interaction between training contest outcome and behavior type (LMM interaction: $F_{1,36} = 3.45$, $p = 0.071$), subjects who won their training contest trended towards showing more dominance behavior than did the subjects that lost their contests (Figure 7).

Training Pair	Winner	dom:sub	% dom	Loser	dom:sub	% dom
1	S1	133:0	100%	AG	4:11	27%
2	S2	28:0	100%	SB	12:7	63%
3	S3	6:1	86%	SB	1:4	20%
4	AG	62:1	98%	S4	46:4	92%
5	S5	88:0	100%	AG	10:9	53%
6	AG	44:0	100%	S6	3:6	33%
7	SB	2:0	100%	S7	0:0	0%
8	SB	18:0	100%	S8	1:0	100%
9	AG	39:1	98%	S9	3:4	43%
10	SB	255:2	99%	S10	47:5	90%

Table 3: Training contest outcomes for Experiment 2. S indicates a subject, AG indicates the aggressive stimulus animal, SB indicates the submissive stimulus animal. The dom:sub ration compares the number of dominant behaviors performed to the number of submissive behaviors performed. The % dominance indicates how many of the performed behaviors were considered dominant

Though the contests were fixed, not all outcomes were expected, in that sometimes the aggressive stimulus lost, or the submissive stimulus won (Table 3). Because of these cases, the subjects were officially assigned as winner- or loser-trained based on the outcome of the training contest, not the predicted outcome.

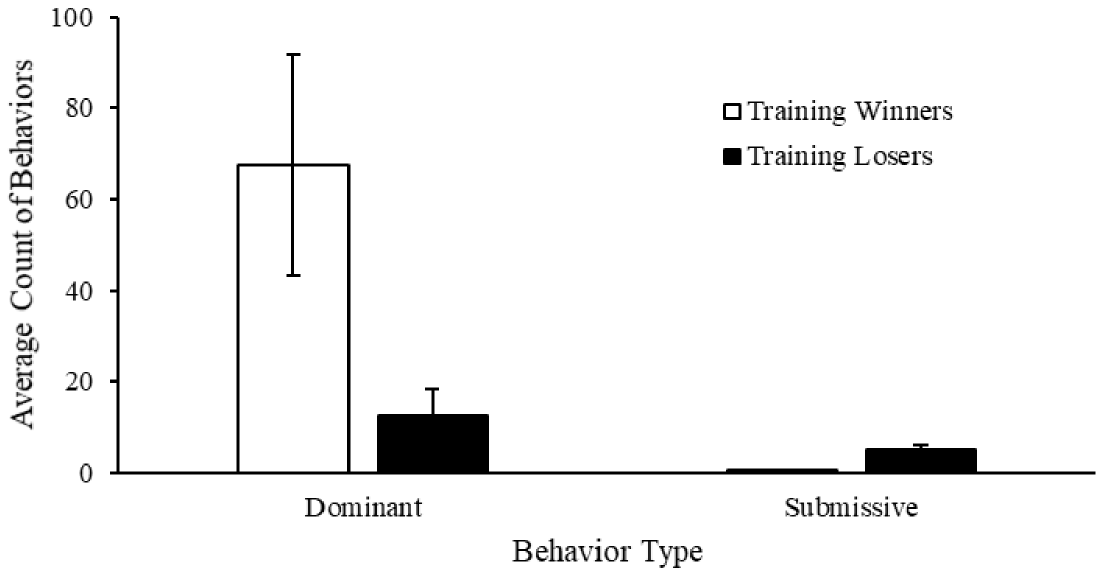

Figure 7: Average number of behaviors in the training contest for winners and losers of the training session in Experiment 2. Error bars indicate standard error of the mean.

Test

Of the 10 contests, three of four subjects who won their training contest also won their test contests, and five of the six subjects who lost their training contest also lost their test contest (Figure 8). Thus, 8 out of 10 subjects showed test contest outcomes consistent with their training contest outcomes, providing evidence for winner- loser effects (Table 4, binomial test, chance =50%; p = .044).

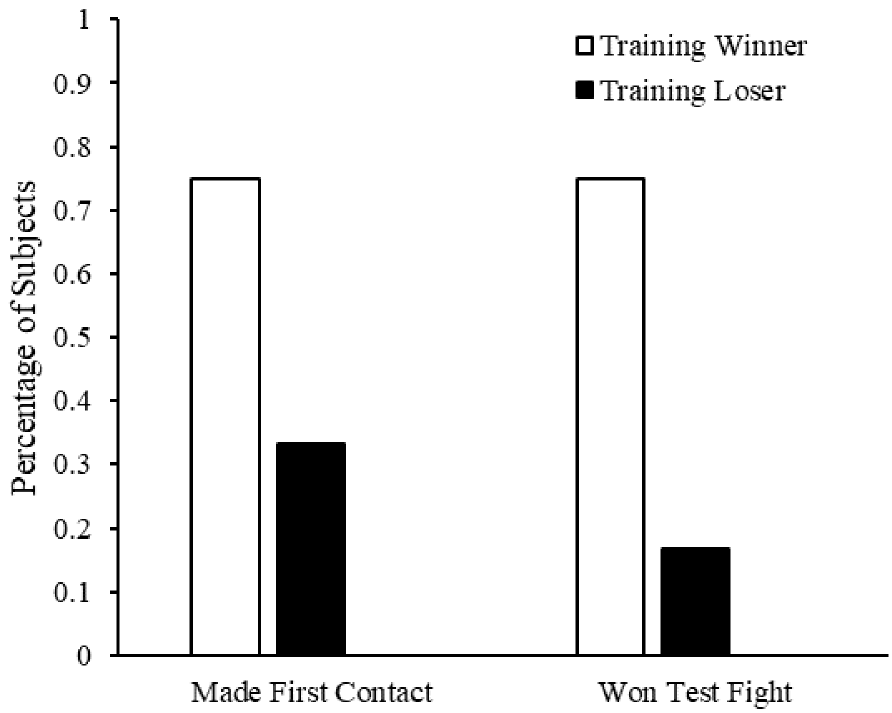

Figure 8: Outcomes for testing broken down by winners, and losers of the training contests. Shown as percentages because of different group sizes.

If previous winning experiences alter an individual's self-assessment of its fighting abilities, subjects that won the training contests should act like they have better fighting abilities than an equally sized naive rival (Hsu et al., 2006), and thus initiate contact more than the rival. Three of four training contest winners initiated first contact during the test contest, while only two of the six training contest losers initiated first contact during the test contest (Figure 8), suggesting that winners may have been more likely to initiate aggression than losers. Interestingly, winners of the test contest initiated the first contact on 8 out of the 10 contests, indicating that the instigator is most likely to be the winner of the contest (Table 4, binomial test, chance =50%; p = .044). The first contact was always a dominant behavior (4 were attacks, 3 were climb ons, and one was approach with antenna contact). This is consistent with previous studies across both vertebrate and invertebrate species which found the first subject to engage in a contest usually won that contest (Hsu et al., 2009; Jackson, 1991).

Training outcome did not have a significant effect on the number of behaviors subjects produced during the test (LMM main effect of training outcome: $F_{1,8} = 1.45$, $p = .263$), and subjects did not show a difference in the number of dominant and submissive behaviors (LMM main effect of behavior type: $F_{1,8} = 2.82$, $p = .131$). Importantly, there was no interaction between training outcome and behavior type on the number of behaviors produced (LMM interaction: $F_{1,8} = 2.06$, $p = .189$), indicating that training winners and losers did not differ in how many dominance or submissive behaviors they produced at test (Figure 9).

Test Pair	Winner					Loser				
	ID	Training Outcome	dom:sub	% dom	First Contact	ID	Training Outcome	dom:sub	% dom	First Contact
1	S1	Win	63:1	98%	Yes	N1	-	9:5	64%	No
2	S2	Win	29:3	91%	No	N2	-	3:8	27%	Yes
3	S3	Win	2:1	67%	Yes	N3	-	0:2	0%	No
4	S4	Loss	22:1	96%	Yes	N4	-	1:1	50%	No
5	N5	-	75:0	100%	Yes	S5	Win	3:2	60%	No
6	N6	-	3:0	100%	Yes	S6	Loss	0:0	0%	No
7	N7	-	38:1	97%	Yes	S7	Loss	2:8	20%	No
8	N8	-	51:3	94%	Yes	S8	Loss	3:20	13%	No
9	N9	-	14:1	93%	Yes	S9	Loss	12:1	92%	No
10	N10	-	8:1	89%	No	S10	Loss	12:2	86%	Yes

Table 4: Results for Test phase of Experiment 2, separated by winners (left) and losers (right). S stands for Subject, N stands for Neural. The dom:sub ratio compares the number of dominant behaviors performed to the number of submissive behaviors performed. The % dominance indicates how many of the performed behaviors were considered dominant

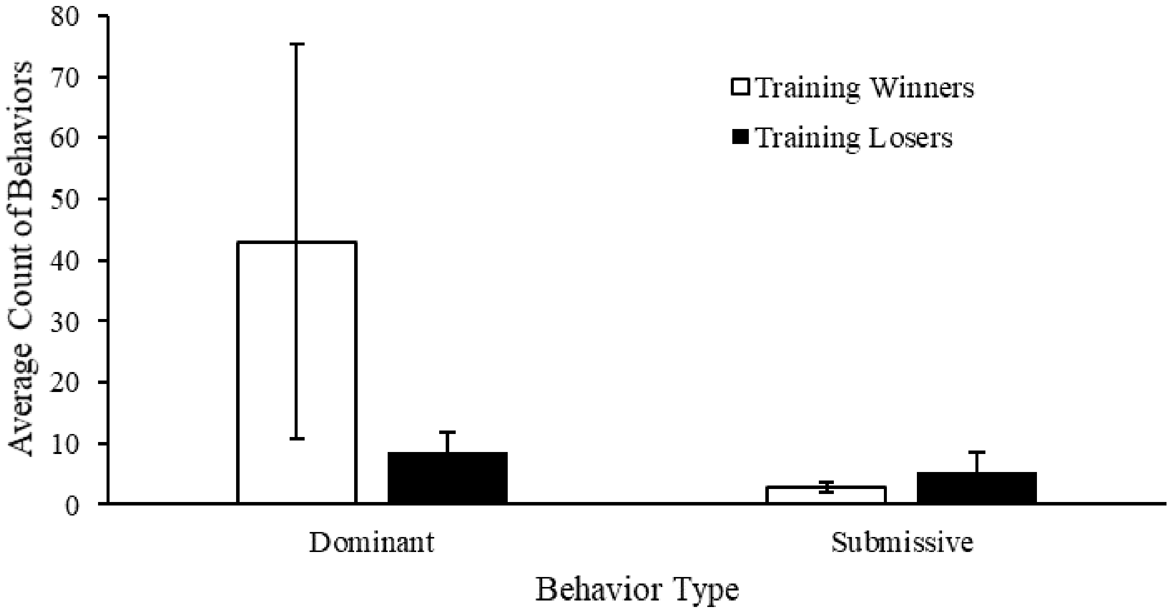

Figure 9: Average number of behaviors in the testing session for winners and losers of the training session in Experiment 2. Error bars represent standard error of the mean.

A Pearson's product-moment correlation compared the training and testing dominance ratios for each subject. This was calculated to investigate whether the amount of dominant actions performed in the training contests were indicative of the amount of dominant actions performed in the test contest. There was no significant correlation between the two (R = 0.504, $p = .137$; Figure 10), indicating that the relative amount of dominance or submission shown by subjects during the training contest did not predict the relative amount of dominance or submission shown by subjects in the test contest.

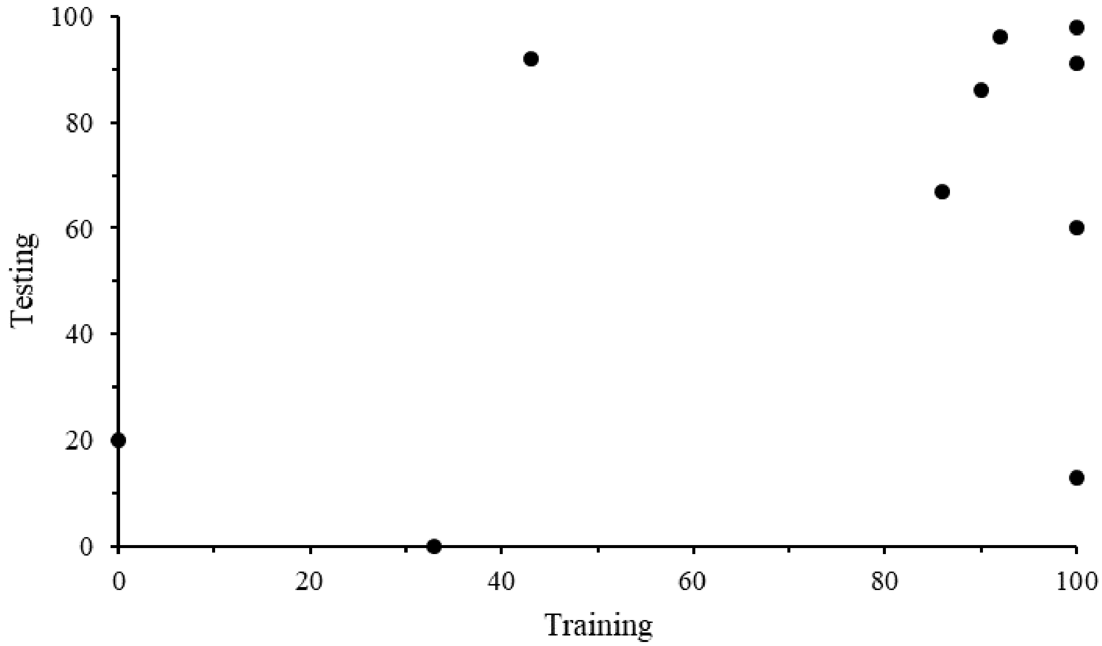

Figure 10: Correlation between training and testing dominance percentages for the 10 subjects in Experiment 2

General Discussion

In Experiment 1, four of the six subjects showed test contest outcomes that were consistent with their training contest outcomes. Likewise, in Experiment 2, when subjects were paired with a naive partner at test, test contest outcomes were consistent with training contest outcomes. Together, these findings suggest the presence of winner and loser effects. Winner and loser effects have been studied thoroughly in other insect species, but have not been investigated extensively in Madagascar hissing cockroaches before this point. The results from this study suggest the presence of both winner and loser effects, because the majority of the training winners won and the majority of the training losers lost their test contests. This differs from some invertebrates, like the olive fruit fly, that increase their aggression in future contests after simply participating in a previous contest, no matter the outcome (Benelli et al., 2015).

Interestingly, in Experiment 2 there was no correlation between the dominance ratios of the training contest and testing contest, indicating that the winner effect or loser effects did not vary systematically based on the intensity of the training contest outcome. Additionally, in Experiments 1 and 2, training contest outcomes did not predict the amount of dominance or submissive behaviors in the test contest, suggesting that if winner and loser effects are present, they did not increase or decrease the number of attacks performed in the next contest. This was inconsistent with previous research, which had found that the number of attacks an individual performed increased after winning a contest, and decreased after losing a contest (Huhman et al., 2003). This may imply that the winner and loser effects only affect the aggression shown in the assessment period at the beginning of an interaction. During a contest, both subjects continuously choose to participate or avoid interactions. So, winner and loser effects may change the probability of an interaction escalating and how a subject gathers information before the contest, but may not change how the subject interacts throughout the contest (Hsu & Wolf, 2001).

The presence of winner and loser effects when subjects were paired against the neutral partners in Experiment 2 provides support for the self-assessment hypothesis as one mechanism behind these effects in this species, primarily by ruling out use of social cuing. Because these competitions were against inexperienced neutral rivals, neutrals would not have systematically displayed social-cues to differentially influence the training contest winner and training contest loser subjects' behavior. The social-cue hypothesis was not supported because if the subjects relied on social cues to assess how to behave during a fight, their training outcomes would not have mattered in Experiment 2. Additionally, aligning with previous research (Breed et al., 1981), eight of ten individuals who won their test contest made the first contact in Experiment 2.

The first contacts were always dominant behaviors, which restricted the behavioral response of the rival because it had to choose a counter behavior. This suggests that when subjects were aggressive, they were aggressive early in the contest, possibly providing additional support for the self assessment hypothesis. The immediate payoff from male-male aggression in this species is critical, yielding access to mates for example, and competitions are not exclusively used to build a hierarchy or maintain dominance relationships (Guerra & Mason, 2005); our results indicate that this payoff also seems to influence future contests.

According to the two-part hypothesis proposed by W. M. Jackson (1991), the winning effect is the product of initiators of aggressive interactions being more likely to win these interactions, as well as to winners being more likely to initiate contests with future rivals. The results of the present study indicate that Madagascar hissing cockroaches exhibit the winner effect, because the winners of contests were usually the individuals who made the first contact, and the winners of the training contests tended to win their testing contest. When using self assessment, aggression displayed during contests stems from subjects reevaluating their chances of winning based on their previous experience winning before engaging in the contests (Hsu et al., 2009). The loser effect appears as essentially the opposite of these two hypotheses, in that the losers were less likely to win (Chase et al., 1994) or initiate their next match (McDonald, Heimstra & Dakot, 1968), which was also seen in this study.

It is possible that winner and loser effects lasted longer than the isolation period of four weeks we used for this study, because how long effects of social interactions last is not yet known in this species. In lobster cockroaches, a similar species that also hisses, the winner and loser effects were found within five weeks of the initial interaction (Kou et al., 2019), but were observed to differing degrees in these five weeks. Most experience effect studies have shown that winner and loser effects last from a few hours, as in juvenile crickets (2 hours; Abe et al., 2021) to a few days, as in Rivulus marmoratus fish (48 hours; Hsu et al., 1999). We therefore think it is unlikely that the results of contests in the present experiment were influenced by the results of social interactions from four weeks prior.

Future Research

Further investigation should be conducted to observe potential attenuation of the winner and loser effects, like increasing or decreasing the delay between the training and test contests (see Chase et al., 1994, Kou et al., 2019, Kasumovic et al., 2010 for methods). Changing the number of training contests could also alter the influence of winner and loser effects. Future research should also explore another phenomenon proposed by Chase et al. (1994): the possibility that the winner effect can cause a subject to win a future contest that it would not normally be expected to win, such as beating a much larger male cockroach in a fight rather than the size-pairing used in this experiment.

Additionally, hormone involvement in winner and loser effects in vertebrates has been studied, as well as in lobster cockroaches (Kou et al., 2019), so it would be interesting to see how hormones affect the outcomes of contests in Madagascar hissing cockroaches. The probability of winning in one cricket species has been found to be influenced by the release of the stimulating and performance-enhancing neurotransmitter octopamine (Benelli et al. 2015). In American cockroaches, aggression, and therefore dominance, can be enhanced by sex pheromones when competing for a mate, in addition to their increased tendency to compete when it comes to territory defense and escape (Bell & Sams, 1983). Our current methods do not allow us to rule out this chemical signal that could affect a rival's assessment of a conspecific.

Ultimately, the results of this study indicate that previous contest winners are more likely to win contests in the future, supporting the presence of winner and loser effects in Madagascar hissing cockroaches. Interestingly, training contest outcomes did not predict dominance or submissive behaviors in test contests, suggesting that winner and loser effects may only affect the aggression shown in the assessment period at the beginning of an interaction. This study suggests that the cause of winner and loser effects stems from the 'self-assessment' adaptive hypothesis, in which winners and losers evaluate their own abilities (rather than reading social cues from opponents of traces of their wins or losses) before competing in a contest. Further, the presence of winner and loser effects in this species suggests that contest outcomes and hierarchy building and maintenance may not solely be based on physical fighting abilities of the individuals, but also other factors that may not be obvious to an outside observer.

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Appendix 1: Ethogram

Madagascar Hissing Cockroach Dominance Ethogram

Behavior	Code	Description
Physical Aggression: Dominance		
Approach	Ap	Walk or run toward another animal. Only antennae contact was made.
Climb on	CO	Climb on top of another animal.
Head Butt	HB	Lower head and rapidly hit other animal with horns
Push	P	Lower head and slowly push on other animal with horns
Attack	At	Repeatedly push, head butt, and approach another individual
Wrestle	Wr	Two animals engage in attack behavior towards one another. Often accompanied by spinning around each other. Neither animal retreats, and both animals must engage in attack/head butting behavior.
Dominant Display Behavior		
Posture	Po	Stand tall, raise head and front of body up, abdomen stays down
Head down	HD	Standing still, lower head below carapace. Similar to the position used in Head butt or push.
Waggle	W	Vigorously shake abdomen
Hiss	H	Emit a hissing sound
Abdominal thrust up	AbTh	Lift lower abdomen up in quick motion
Submissive Behavior		
Retreat/Withdraw	Wd	Walk or run away from an approaching animal
Freeze	F	Lower head and body, do not move
Side tilt	ST	Lower head, lean body toward other animal

Appendix 1: Ethogram used to record behaviors during training and testing contests

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