

Eat, Prey, Love:
Sex influences nest intruder behavior in the maritime earwig
(*Anisolabis maritima*)

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*****DISCLAIMER: This data contained in this paper were collected and analyzed during an 8-week REU program; the results will be re-analyzed and further explored in preparation for future publication in a peer-reviewed journal. Please contact the PI (vikram.iyengar@villanova.edu) if you have any questions about this paper or the data contained herein.**

Abstract

The most common form of parental care in insects is the guarding of eggs/juveniles from intruders. The maritime earwig (*Anisolabis maritima*), an insect found on beaches worldwide, is an excellent organism for studying parental care because they are found in high densities under driftwood during the breeding season. As a result, egg-guarding mothers encounter many types of intruders and are especially threatened by conspecifics that often cannibalize eggs. We studied how sex affects nest intruder behavior by monitoring the behavior of size-matched intruders of different sexes in an arena with a mother and her eggs over twelve hours. The time-lapse videos were then uploaded to BORIS behavioral scoring software, where we scored the number and durations of invasions, cohabitations, attacks, courtships, and matings. We found many differences in behavior based on the sex of the intruder, as males invaded the nest more frequently and remained in closer proximity despite vigorous nest defense by the mother. Overall, we found that female intruders were much less aggressive than predicted, and that male persistence to return to the female's shelter is key to receiving a high number of matings. Our study is the first to monitor these intimate interactions in the maritime earwig, and our results suggest that mothers may protect their eggs and appease male intruders by mating with them.

Introduction

Parental care, defined as any investment by one or both parents that benefits their offspring (Trivers 1972), has evolved in a wide array of taxa, including mammals, birds, fish, reptiles, amphibians, crustaceans, arachnids, myriapods, and insects (Douglas 1984). Parental care likely evolved due to harsh environments, high rates of parasitism or predation, and increased conspecific competition selecting for attentive parents that provided care to their offspring and increased their likelihood of reaching maturity (Ross 2022). The guarding of eggs and early-stage nymphs is the most common form of parental care in insects, and most likely evolved due to predation pressure (Costa 2006; Suzuki 2010). Intruders are thought to invade nests to practice oophagy, or egg eating, because eggs can be an easy and high calorie meal. Male intruders may have additional motivation to practice oophagy, as eating a female's eggs may induce the mother into becoming reproductively receptive once more, as seen in mammals such as lions (Bertram 1975), primates (Hrdy 1976), and rodents (Mennella and Moltz 1988).

When parental care involves males guarding either food, eggs, or mates and/or females guarding eggs or nymphs, the parents exhibit territoriality in that they are defending a specific area from intruders (Baker 1983; Ross 2022). Territorial disputes are often won by the individual with the highest resource holding power (RHP), defined as an animal's ability to obtain or keep a resource (Parker 1974; Ross 2022). When two opponents face off in battle, there is often asymmetry in RHP (Hurd 2006) due to individual differences in morphology, motivation, resident status, and previous experience (Dugatkin 2000). Differences in morphological features such as size and weaponry create asymmetries in RHP that influence success in aggressive interactions (Vieria and Peixoto 2013; Ross 2022). Similarly, interactions between a resident holding a resource and an intruder may have motivational asymmetries that influence RHP and,

thus, success (Ross 2022), with the resident typically having more motivation to keep the resource (Parker 1974). Evidence suggests that intruders are less motivated and less likely to win aggressive battles with a resident (Chapin and Hill-Lindsay 2016). We would expect to see such increased motivation by residents when they are parents defending their offspring.

Study system

Maritime earwigs (*Anisolabis maritima*) are members of the Family Anisolabididae that are found on temperate and tropical coasts around the world (Hincks 1947). Their preferred habitat is underneath driftwood and debris, typically within 5 meters of the high tide line on beaches (Bennett 1904; Hack and Iyengar 2017). Maritime earwigs are sexually dimorphic in both morphology and temperament; females use their scissor-like forceps to vigorously defend their nests from conspecifics and to ward off unwanted matings, while males use their curved forceps to squeeze the abdomens of other males in ritualistic battle (Figure 1; Ross 2022). Females are the more aggressive sex, likely due to exclusive maternal care when surrounded by high densities of cannibalistic conspecifics (Ross 2022; Dodgen and Iyengar 2015).



Figure 1. Male and female *Anisolabis maritima*

Females lay an average of 42 eggs (Miller and Zink 2011) in sand-divot nests underneath driftwood in May through August (Miller and Zink 2012). Mother maritime earwigs perform a variety of parental care behaviors such as grooming and sorting the eggs and viciously defending

the nests (Ross 2022). Intruders, defined as non-kin conspecifics that invade a female's nest, likely do so to either make a meal out of the eggs, mate with the female, or both.

We hypothesized that there will be sex-based differences in intruder behavior, likely due to the additional reproductive motivation of males. We predicted that male intruders would have higher instances of nest invasion and attempt to invade the nests faster to eat the eggs and allow the female to become reproductively active once more. We also expected male intruders to have more instances and longer durations of cohabitations with the mother because being in her presence increases their probability of mating. Additionally, we predicted that female intruders would attack the mother on more occasions and for longer durations, as well as be more likely to expel the mother from her nest due to their increased aggression and desire to take over the mother's nesting area.

Methods

Experimental Design

This research was conducted at Friday Harbor Laboratories (FHL) on San Juan Island, Washington for 8 weeks in June-August of 2022. We hand-collected *A. maritima* to act as intruders as well as mothers and their nests from the following sites: False Bay, Argyle Lagoon, Cattle Point, and Friday Harbor Labs. Future intruders were collected and transported in plastic medicine vials, and they were eventually placed in individual glass jars (0.5L, with 0.5 cm sand) and assigned an identity based on the field site they were collected from and a number (ex: FB150, for the 150th individual collected at False Bay). We collected egg-guarding mothers in the field for use in our experiments. In these cases, we collected the mother, used a soup spoon to scoop the eggs (and some sand) into a small, translucent red shelter (3.5x3.5x2 in), and then

placed mother and the egg-containing shelter into a glass jar (0.5L) with 0.5 cm of sand. All individuals – both intruders and mothers – were photographed beside a ruler and ImageJ was used to measure their pronotums to size-match them to the mother (Tsao 2021).

Each of the trials was conducted in a plastic bin (15x19x7 in) that contained a 0.5 cm thick layer of moistened sand and plexiglass boards to simulate the underside of driftwood (Ross 2022). Translucent red acrylic paper was placed on each of the plexiglass boards to create a dark habitat for the *A. maritima* underneath the boards due to their inability to see red light, while allowing us to witness their behavior. After the mother laid all her eggs (i.e., she did not lay any additional eggs over 48 hours), we moved her (and the red shelter containing her eggs) to the trial bin, where we covered her and her eggs with a larger plastic container (5x5.25x2.6 in) to act as an isolation chamber (Fig. 2).



Figure 2. An arena with a mother inside the isolation chamber and a female intruder with a dot of nail polish to distinguish her from the mother. Inside the isolation chamber, the mother is guarding her eggs in the shelter.

To control for size, we selected intruders that were the same size as the guarding mother, and we used the criterion of our size-matched intruder having a pronotum width of $\pm 5\%$ of the guarding mother. Female intruders were given a small dot of white nail polish on their pronotum to distinguish them from the mother, whereas males did not need any such markings since we could distinguish them by the shape of their forceps (Ross 2022). The intruder was placed in the trial bin while the mother was in the isolation chamber for eight hours so they could acclimate before the trial began (Ross 2022). The trials were recorded using a mounted GoPro HERO10 Black, which took a photo every two seconds for the 12-hour trials (Ross 2022). The trials began every night at around 8:00 PM PDT and ended every morning at around 8:00 AM PDT.

Data Analysis

The videos from the GoPro were uploaded to an external hard drive, where we processed and recorded their behaviors using BORIS behavioral scoring software. Specifically, we noted the timestamp and duration of attacks (when the mother and intruder struck each other's abdomens with their forceps), invasions (when the intruder had over 3/4ths of their body in the shelter), courtships (when males waved their forceps in the mother's face), matings (when the male and mother sat in the classic "forceps-to-forceps" mating position), and cohabitation (when both individuals were in the shelter and the intruder was relatively still). Intruder "success" was defined in multiple ways, such as if the intruder inhabited the shelter at the end of the trial, if the intruder ever forced the mother from the shelter, and if any eggs were eaten.

We used t-tests to analyze the effect sex had on time until the first encounter, interval between the first and second encounter, and total number and duration of attacks, invasions, and cohabitations. We used a 2-way ANOVA test to ascertain the effect sex and success rate had on the factors previously described. We also used a Chi-square goodness-of-fit test to determine the

effect sex had on intruder success, and a Chi-square test-of-independence to quantify intruder success between the sexes.

Results

We found a significant difference in the number of individuals that invaded the shelter based on sex, with males being more likely to invade the shelter (Table 1: $X^2 = 4.86$, $df = 1$, $p = 0.028$).

We also found a significant difference in the number of individuals who invaded a second time, with males returning to the shelter every time (100% of all trials) whereas females only returned 37.5% of the time (Table 1: $X^2 = 9.29$, $df = 1$, $p = 0.002$). However, there was no difference in the number of individuals who forced the mother from the shelter based on sex, with both sexes only having one intruder (of the 8 total per sex) successfully forcing the mother from her nest.

Males invaded the shelter 5 more times on average than the females (Figure 3: male $\bar{x} = 18.75$ vs, female $\bar{x} = 3.63$), and have a four-fold longer duration of invasion (male $\bar{x} = 237.28$ vs. female $\bar{x} = 55.37$; $t = 2.37$, $df = 14$, $p = 0.0329$).

Table 1. Invasion of nests by males and females (n=8 trials for each). Males were more likely to invade the shelter, and both sexes were equally unlikely to force the mother from the shelter.

	# of individuals that invaded the shelter	# of individuals that invaded a second time	# of individuals that forced the mother from the shelter
Males	8/8	8/8	1/8
Female	5/8	3/8	1/8
Chi-square test (p-value)	0.028	0.002	1.00

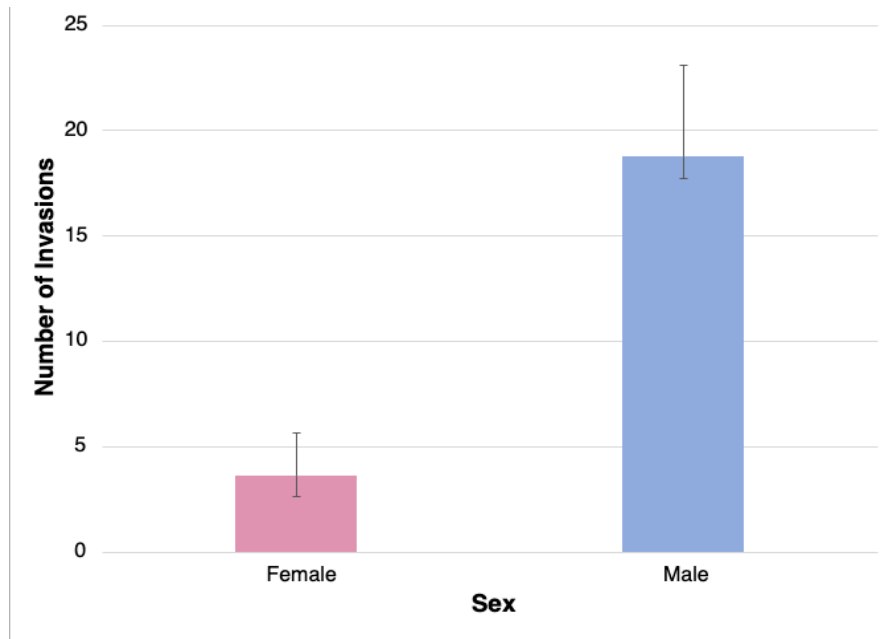


Figure 3. Males invaded the mother’s nest five times more often than females ($t = 3.15$, $df = 14$, $p = 0.007$).

There was no significant difference in number of attacks based on sex (Figure 4: male $\bar{x} = 38.5$ vs. female $\bar{x} = 7.13$). However, males had 16 times more cohabitation events than females (male $\bar{x} = 8.13$ vs. female $\bar{x} = 0.5$; $t = 2.37$, $df = 14$, $p = 0.033$), and remained cohabitating 13 times longer than females (Figure 5: male $\bar{x} = 132.41$ vs. female $\bar{x} = 10.2$; $t = 2.48$, $df = 14$, $p = 0.026$). We also found that, although the result was not significant, there was a positive *trend* between the number of attacks by the males and the number of reproductive behaviors (matings + courtships) allowed by the egg-guarding mother (Figure 6).

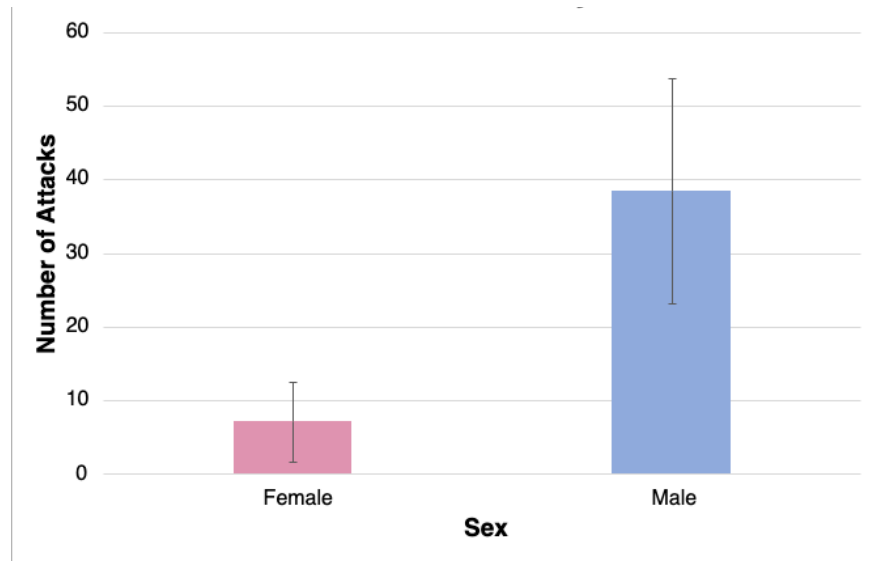


Figure 4. There was no difference in the number of intruder attacks on egg-guarding mothers based on sex, although there was a *trend* that males attacked more often than females ($t = 1.93$, $df = 14$, $p = 0.074$).

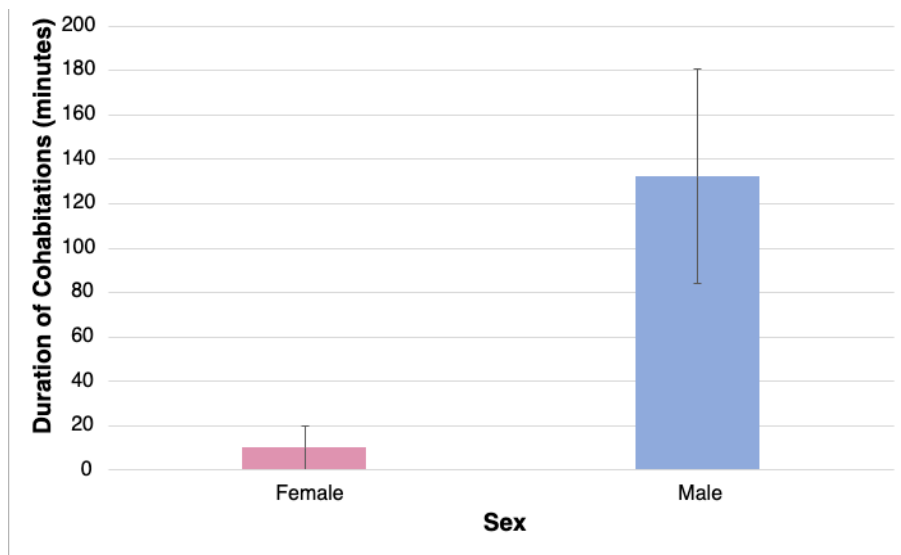


Figure 5. Males cohabitated with egg-guarding mothers over 13 times longer than females ($t = 2.48$, $df = 14$, $p = 0.026$).

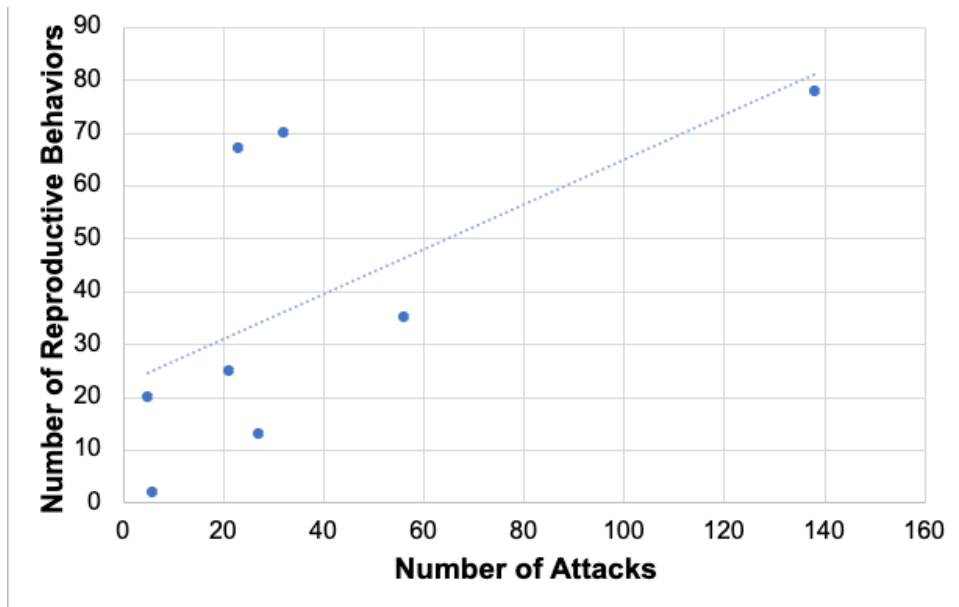


Figure 6. Although this result was not significant, there was a positive *trend* between the number of attacks by the males and the number of reproductive behaviors (matings + courtships) allowed by the egg-guarding mother ($F = 4.01$, $df = 1,6$, $p = 0.092$, $R^2 = 0.40$).

Discussion

In this study, we found differences in nest intruder behavior based on sex that were likely due to the additional reproductive motivation of males. Out of the eight male trials, all of the male intruders invaded the female's shelter at least twice. Males typically invaded the nests within the first hour of the trial and, although these encounters often involved aggressive attacks, male intruders cohabitated significantly longer than female intruders with the egg-guarding mothers.

Females were surprisingly less aggressive towards the mother and her eggs than we predicted. Only five females of the eight invaded the shelter once, and only three of the five invaded the shelter a second time. In both male and female trials, only one of the eight intruders was able to force the mother from the shelter with an attack. This result reflects the mother's

high motivation to protect her offspring and win these territorial battles for the nesting area. We found a surprising (though non-significant) result in that females averaged only 7 attacks in 12 hours, compared to nearly 40 for males. Overall, we found that female intruders were much less aggressive than predicted and often opted to leave the mother and her eggs alone after failing to take the nest from the mother. In other words, for female intruders, the motivation of taking over the nest box is not a strong one, nor is the urge to cannibalize the eggs.

One of the most interesting takeaways from this study is that male persistence is key to increased mating success. Males, who repeatedly returned to the shelter even in the face of attacks, seemed to gain more mating opportunities with the egg-guarding mother. When comparing the number of attacks with the number of courtships and mating combined, there is a positive, but not significant, correlation. This pattern is supported by previous work showing that males who sustained more attacks were more likely to receive mating opportunities (Nolan-Tamariz 2016). Based on our results, it appears that mothers to use matings to appease the male and distract him from her eggs, thereby protecting her current nest of eggs from predation and potentially increasing her reproductive output overall. Another student in the Iyengar lab is currently researching the mother's behavior towards nest intruders of different sexes and sizes, and her data will help confirm this hypothesis (Ross 2022).

Please note that the results reported in this paper are incomplete, as our data need to be re-analyzed. We plan to review and rescore the videos to incorporate some additional factors; we will then re-do our statistical analyses and present our results at a future conference and in a peer-reviewed publication.

Conclusion

Overall, this study found that there were differences in nest intruder behavior based on sex. Males had a significantly higher number of individuals who invaded the shelter, returned to the shelter, total number of invasions, and duration of cohabitations. Females were much less aggressive than predicted, and often left the mother and her eggs alone after they were forced from the shelter, if they invaded at all. Males, who kept coming back to the shelter even when faced with attacks, were rewarded for their persistence by mating opportunities. In the future, this study could be improved by having a larger sample size and incorporating different-sized intruders.

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