

Roles of Stridulation in *Nicrophorus investigator*

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Abstract

Stridulation is a key behavior to determining survival in *Nicrophorus investigator*. In this paper, the roles it plays in competition over a carcass, burying process, and larval development have been studied. Their ability to stridulate was manipulated through clipping part of their plectra and they were given simulations that required some form of communication. Stridulation is vital to effectively communicate certain dominate behaviors. The experiments suggests that it is necessary to decrease the time it takes to winning a carcass in order to complete the beginning stages of the Nicrophorine life cycle.

Introduction

One of the many forms of communication for insects is stridulation. Stridulation is the movement of the *pars stridens* on the *plectra*, causing pulses of sound. Although it is unknown if it is heard or if the vibrations from the sound are felt by certain insects, it has been suggested that it is used for communication during competition over a food source or mating (Darwin, 1871; Cai, 2014). The burying beetle is one of many species of carrion beetle found in Western North America (Scott 1998) that are known to stridulate.

There is still some ambiguity as to what purpose stridulation serves. In Hall's experiments, she clipped the *plectra*, or the wing casing, which "mutes" stridulation in many Nicrophorine species (2013, 2015). She has found that this will change the characteristics of the pulses in the sound. However, she has found that the beetles will mate without regard to their ability to stridulate. However, if the female partner or both partners were muted, there were

significantly fewer larvae than an unclipped female partner or if both partners were unclipped (2015).

Stridulation has been observed during many phases of *Nicrophorus* reproduction, including fights at carcasses, the burial process, mating, and larval rearing (Rosemary Smith and Vivian Ortiz's personal observation). In this project, I investigated four different phases in the pre-larval and post-larval stages. The specific questions I addressed were:

- 1.) Does stridulation play a role in determining who wins a carcass?
- 2.) Does stridulation enhance coordination in carcass movement and burial process?
- 3.) Does stridulation influence the ability of newly hatched larvae to locate the carcass?
- 4.) Does stridulation improve larval feeding and growth?

Methods

This study was conducted at the Rocky Mountain Biological Laboratory in Colorado. To trap beetles, coffee cans were filled with about a third of dirt, a frozen chicken drumstick or mouse was placed on top of the dirt and some water was poured on top to moisten dirt. Ten cans were tied to aspen trees about 1.5 meters high and 30 meters apart. There were two sets of traps, one placed northwest of Gothic and the other placed northeast of Gothic (Figure 1). Cans were checked daily and all *N. investigator* were collected and returned to the captive beetle facility where they were watered and fed daily.

Phase 1: Male versus Male Competition

This phase consisted of two trials. Both trials consisted of three treatments: muted vs non-muted, muted vs muted, and non-muted vs non-muted. To mute the beetles, their inner

elytron was clipped in a “V” shape. Non-muted beetles received a sham clipping on the outside of their elytron, which did not hinder the ability to stridulate (Hall 2015). Pairs were assigned by similar pronotum width of less than 10% difference because pronotal width determines the “winner” of a carcass (Safryn 2000). Then they received a randomized treatment using a random number generator. Using a quarter, each pair randomly received either a pink or blue marking on their pronotum to distinguish the two males and reduce observer bias. In trial one, the males were placed in the tubs.

In the second trial, mass before and after the competition was recorded. A female investigator and a lab mouse were placed in a measurement x measurement x measurement tub 24 hours before the experiment began and were replaced by the males.

For both trials, observations were made daily for 3 days to see whether the pink or blue male was on the carcass. The color that was seen on the carcass the most at the end of the 3-day period, was declared the winner. Losers were removed and individually placed in a measurement x measurement x measurement coordination/breeding tubs with a new mouse for the next experiment. Winners kept their mouse and were also placed individually in measurement x measurement x measurement coordination/breeding tubs.

Female-Male Coordination

There were four treatments: mute male and mute female, mute male and sound female, sound male and mute female, sound male and sound female. In the first trial, a breeding box that was half filled with dirt, was placed in a large tub. A 3x5 inch piece of chicken wire lead from the bottom of the tub to the breeding box, acting as a ramp. A pair (n=6) from each treatment was placed at the bottom of the ramp with a mouse. The goal was for the pair to drag the carcass

up the ramp and into the breeding box. However, after 3 days of no movement towards the ramp, the pair and mouse were placed into the breeding box to move onto the next phase.

In the second trial, a 3x2 inch chicken wire was placed in the breeding box on top of the dirt and a mouse was placed on the wire. A pair (n=9) from each treatment was placed into a box. The goal was to have them move the mouse from the wire and into the dirt but after 4 days, only a five out of 48 moved the mouse. The wire was then removed and the beetles continued onto the next phase.

Treatment	Winners	Losers
Mute vs Mute	2	3
Mute vs Sound	3	3
Sound vs Sound	3	3

Table 1 *Trial 1* The male vs male competition had two sets of ties, Mute vs Mute and Sound vs Sound, and one indistinct outcome, the Sound vs Sound.

*Female-larvae Development****

***I am waiting to receive results from the final phase of my experiment. I will have data for the final phases by the end of this week. I plan on finalizing the results before I leave August 20th.

Results

Phase 1 Male vs Male competition

For the first phase, the wins and losses between the groups of treatment were compared (table 1) using a Matched Pair Test through JMP 12. There was no significance in either treatment, Mute vs Mute ($p=.65$), Mute vs Sound and Sound vs Sound ($p=1.0$).

However, when the female was placed for 24 hours prior to the males, there was more activity (figure 2). Although Mute vs Mute ($p=.47$) and Sound vs Sound ($p=.25$) had insignificant results, there was a strong trend for the Mute vs Sound (.08). This suggests that muted beetles are less likely to win than beetles that are able to stridulate.

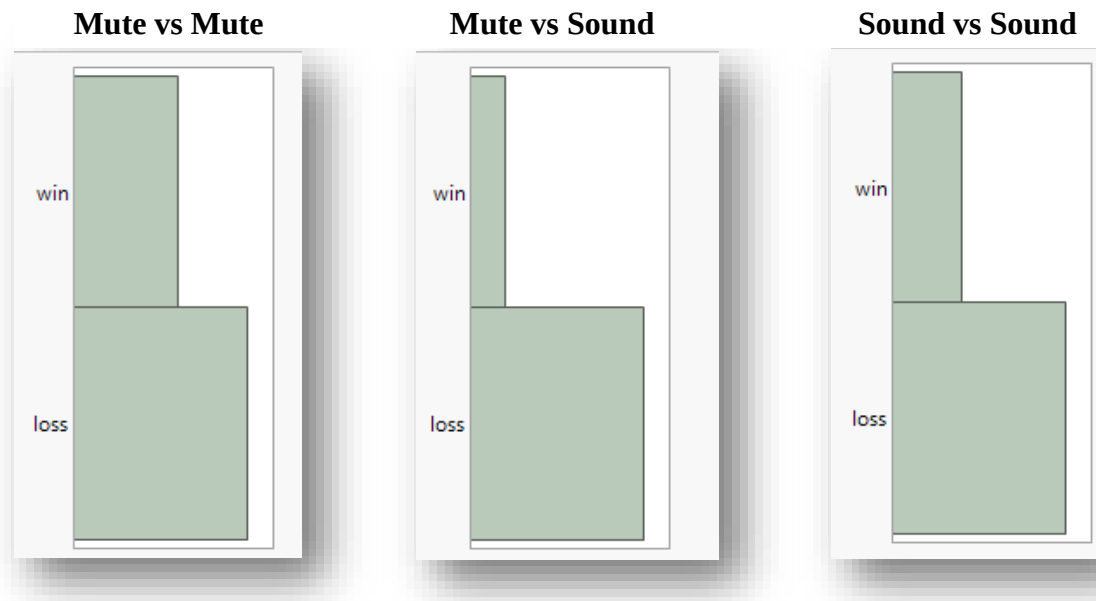


Figure 2 Trial 2 These are the wins and losses from each treatment, showing more activity with a female's scent. When looking at the Mute vs Sound pairs, it suggests that stridulation is needed to win carcass.

Phase 2 Female-Male Coordination

An ANOVA test was ran to compare how long it took for each treated pair to lay eggs ($p=.39$) and for the first larvae sighted ($p=.98$) (Figure 3). Although it was insignificant, the days

to eggs for the Muted pair and Sound pair were compared using ANOVA ($p=.03$). A bar graph comparing the days to eggs and days to larvae shows that it took two days longer for the muted pair to lay eggs than the sound pair and that eggs were less likely to hatch if muted (Figure 4).

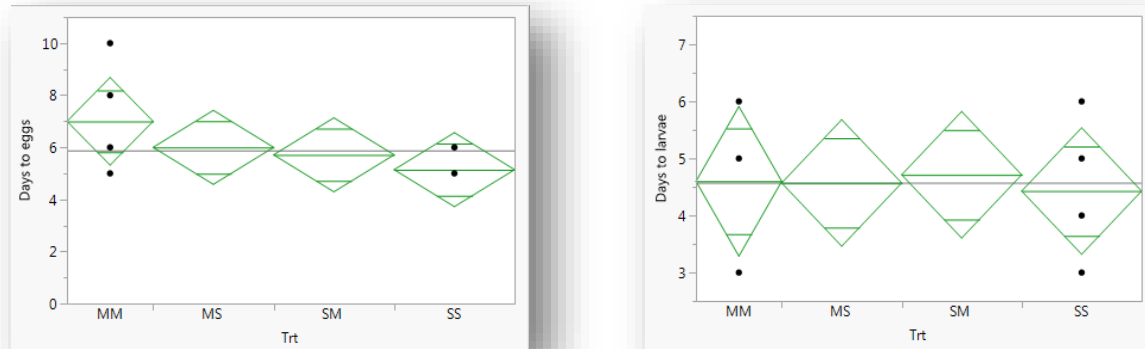


Figure 3 ANOVA test showing the days to eggs (left) and days to larvae (right). Treatments were denoted as MM= Mute pair, MS= Muted male and Sound female, SM= Sound male and Muted female, and SS=Sound pair.

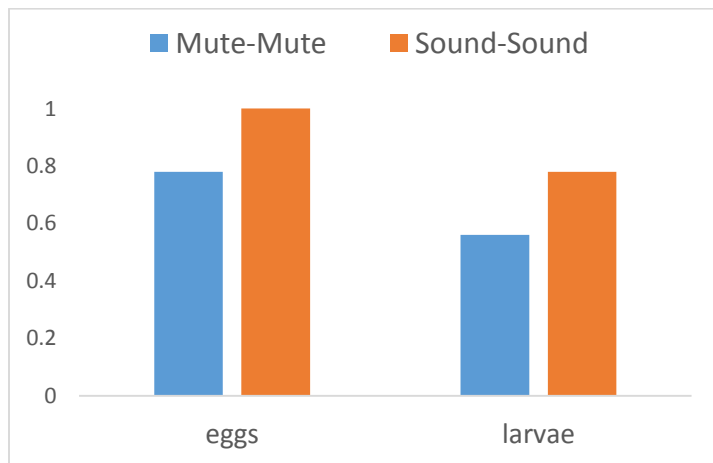


Figure 4 Bar graph comparing the number of days it took to lay eggs and the number of days it took for the first larvae sighted between the muted pair and sound pair.

Discussion

Although the roles of stridulation are unknown, my project tested the importance in vital behaviors such as competition over a carcass, mating, coordination between partners, and communication to larvae. Muting beetles served as a way to hinder their ability to create the sound pulses and it proved that in some scenarios, the ability to stridulate was key to being the most fit for survival.

In the first phase of the experiment, the presence of female scent was important for motivating the male vs male competitive interactions. If the males were muted, they were less likely to win a carcass. In the second phase, I found that if pairs were both muted, it took about two days longer for them to lay eggs compared to the sound pairs. Also, if the pairs were muted, they were less likely to lay eggs and rear larvae than the sound pair.

In nature, it is vital to find a carcass that can support a female and larvae to pass on genes, therefore, stridulation is necessary to warding off competitors. It was thought that having the largest pronotal width always determined the winner of a carcass (Safryn and Scott 2000). Perhaps it's not always about size that declares a beetle as the "fittest". The study also found that the inability to stridulate delayed egg-laying time. The longer it takes to reproduce, the more they risk losing the carcass to predators. Perhaps stridulation is also a key factor to determining the outcome of survival.

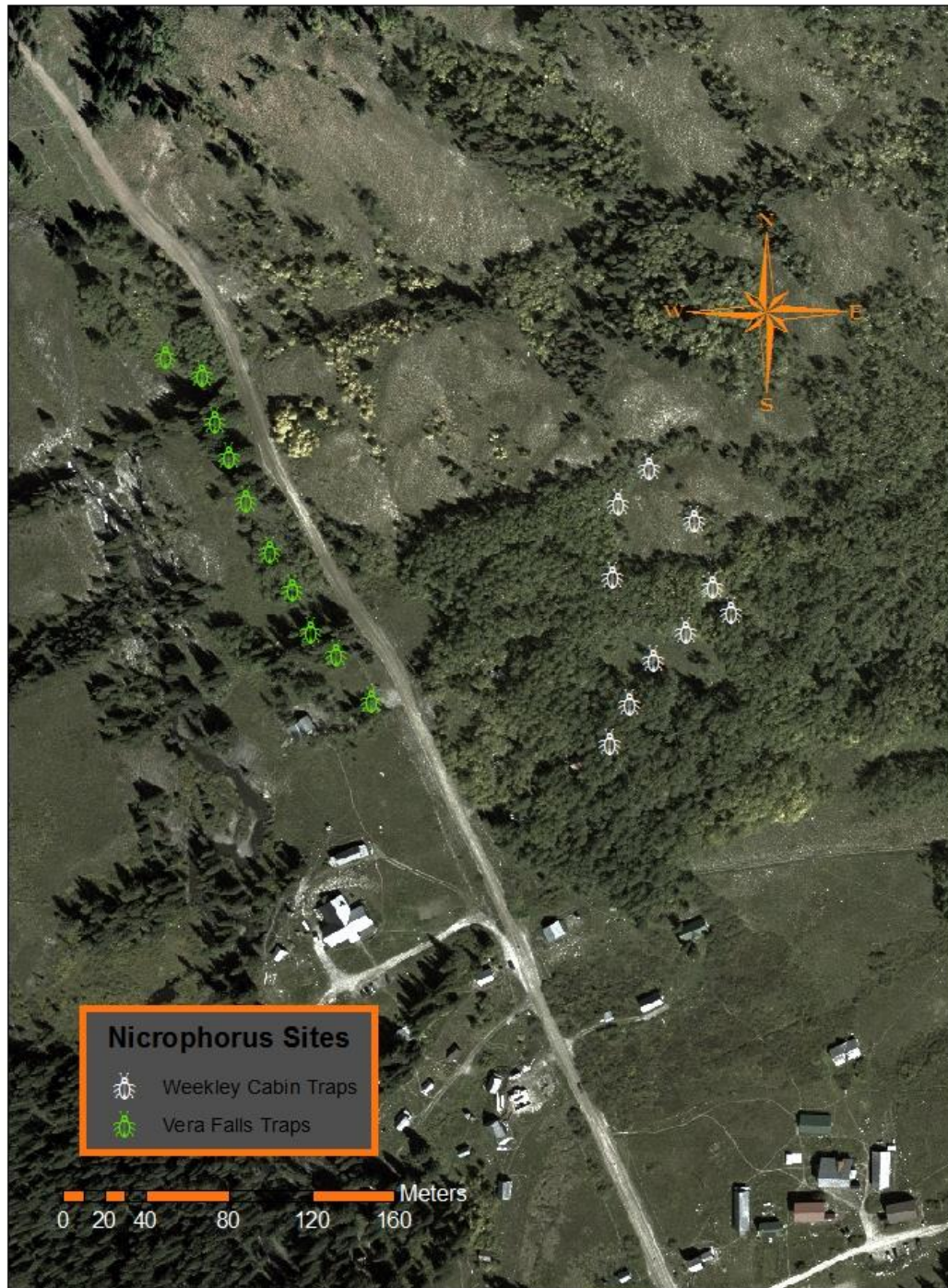


Figure 1 This is a map of the gothic town site. The beetle traps are shown above.

Literature Cited

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