

Stridulation in *Nicrophorus investigator* as a Possible Means of Cooperation during Carcass Sequestration

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Abstract

Burying beetle stridulation has been observed and documented when caring for their young, as well as among agonistic beetles competing for carrion. Burying beetles in the genus *Nicrophorus* require a small carcass, typically 16-50 grams, in order to reproduce. Once located, the carcass is buried by the burying beetles, after which the beetles lay their eggs in the underground chamber and partake in biparental care. This study found that *N. investigator* pairs use stridulation during carcass sequestration when faced with an obstacle, but found no significant difference between the beetles' use of stridulation and lack thereof in terms of their ability to move carrion.

Introduction

Communication can be described as an association between the behaviors of a sender and a receiver as a consequence of a signal (Wiley and Richards, 1978), which is designed to influence the behavior of listeners by transmitting information (Seyfarth and Cheney, 2003). Natural selection seems to favor those individuals that can vocalize to affect the listener's behavior, as well as those individuals that can obtain information from those vocalizations. (Seyfarth and Cheney, 2003), but detection of these acoustic communications require the receiver to have the ability to not only hear the signal, but to also be able to discern between distinct signals (Wiley and Richards, 1978).

Insects are known to produce audible cues. Whether its crickets, cicadas, grasshoppers, or other audible insects, the sounds produced may be due to a disturbance, or a distress in the insect, or sometimes for courtship and mating, and at other times used to warn or intimidate other insects (Alexander, 1957). In *Nicrophorus* beetles, stridulation is known to be used when rearing young (Milne and Milne, 1976), when fighting (Milne and Milne, 1944), and during mating (Pukowski, 1933).

Stridulation in *Nicrophorus* beetles is produced when the plectrum (a scraper located on the ventral side of the elytra) is moved across the surface of the pars stridens (a rigid file of individual teeth located, from the caudal end, on the fifth abdominal segment in males, and fourth segment in females) (Hall et al., 2013, 2015). Stridulation is suggested to play a more important role in behavioral contexts such as competition for resources of reproduction than as a call song (Hall et al., 2013). Examples of its use can be seen when stridulation is used to attract the larvae to the adults when in their underground chamber in order to feed them regurgitated carrion (Milne and Milne, 1976), although it was found that sound production played a more important role in brood success for females than males (Hall et al., 2015), and it was observed in *N. orbicollis* when expelling competitors, as well as when confronted with an obstruction

blocks the path of the beetle during sequestration of the carrion (Milne and Milne, 1944). It may be possible that the stridulation observed by Milne and Milne from *N. orbicollis* when faced with an obstacle along their path during carrion sequestration was made as a means to communicate with other beetles and recruit their efforts to bypass the obstacle. It's possible that other *Nicrophorus* beetles might engage in this same patterns of behavior when faced with an obstacle of their own, and so, this experiment hoped to extend that observation to *N. investigator* and further our understanding of communication and cooperation in these beetles.

To do so, I created a confined scenario that would force the beetle pairs to carry a mouse carcass a certain distance, as well as having to overcome an obstacle (see fig. 5). Secondly, I experimentally manipulated sound production in *N. investigator* pairs to see if it had an effect on carcass sequestration.

Methods

Study Organisms

Nicrophorus investigator was chosen as the main study organism due its relative abundance compared to the other native *Nicrophorus* species, *N. defodiens*, *N. guttula*, and the rare *N. marginatus*, found in Gothic, Colorado. This species is diurnal (Smith et al., 2000b) and has a preference for warm meadows, where sunny conditions accelerate larval development (Smith and Heese, 1995).

Nicrophorus Beetles

The *Nicrophorus* genus of beetles is found in the northern hemisphere and consists of about 75 species (Scott, 1998). The beetles within this genus depend solely on the presence of small carcasses for reproduction. Commonly known as “burying beetles,” for their burial of carrion for reproductive purposes, these beetles are also known for their extended biparental care of their young (Scott, 1998).

Detection of the carrion by *Nicrophorus* beetles is accomplished via chemoreceptors found on their antennae, which detect the sulfur-containing volatiles produced from dead organisms (Kalinová et al., 2009). Once detected, the beetle may begin walking around the vicinity, perhaps in search of a viable substrate in which they can bury the carrion (Milne and Milne 1944, Smith et al., 2000a). Different *Nicrophorus* spp. have been shown to prefer soil with added structural bulk, such as grass, or leaves (Muths, 1991), or in some cases use of already existing holes (Smith et al., 2000a) was preferred, as both of these methods allow for faster burials. All these can prove to be essential in cases where competition is abundant (Muths 1991, Lomolino and Creighton, 1996). *Nicrophorus investigator* also assess the carcass's weight, as it was shown to have a preference for carrion within 16-48 grams (Smith and Heese 1995, Smith and Merrick, 2001). It's possible that lighter than 16 grams may not be enough to feed the brood, and be therefore not worth the beetles' time, while heavier than 50 grams may be more difficult to handle and sequester (Trumbo, 1990).

Carcass Sequestration in *Nicrophorus investigator*

The process of carcass sequestration in *N. investigator* begins by the beetle positioning itself upside-down underneath the carcass, such that its legs face upwards, making contact with the carcass, while its dorsal side is touching the ground. It then uses its legs to push the carcass towards the site of burial (Milne and Milne 1944, 1976). This process may be started by one beetle, a pair of beetles, or more if several beetles find themselves at the site of the carrion within the same timeframe; however, only one pair will ultimately claim the carcass for itself and reproduce once sequestration is complete (Milne and Milne, 1976, Wilson et al., 1984). In those cases where intraspecific conflict occurs between *Nicrophorus* beetles, size is usually a dominating factor (Otronen, 1988). Once that pair has fought off the other beetles, the carcass is buried, curled into a ball, and covered with anal and oral secretions to prevent development of bacteria and fungi (Hoback et al., 2004). For *N. investigator* in Colorado, the eggs are laid in the surrounding soil and hatch about 56 hours later (Solis and Smith, 2017). The parents attend the brood for 12-18 days, until the carrion has been consumed by the larvae, at which point the adults leave. The larvae then overwinter as prepupae, before pupating the following summer and eclosing as adults in mid to late June (Smith et al., 2000b).

Abandonment of the carcass may occur if the competition from flies, ants, other beetles, and bacterial and fungal decomposers is too great (Hoback et al., 2004). A previous study showed that while *Nicrophorus investigator* took about 2 days to reach a carcass, flies and ants made their way within 1 to 2 (Smith and Heese, 1995). And while burying beetles may be able to combat dipteran competitors by either directly destroying the fly eggs/larvae, or using the mutualistic phoretic mites that live on them to do the same, excessive competition from flies can lead to the abandonment of the partially buried fly larvae ridden carcass (Milne and Milne, 1944), which further highlights the importance of a speedy burial, since a quick one would reduce the amount of time the carcass is exposed and therefore reduce the competition from flies. Previous studies have looked into the effect of multiple pairs on burial speed for other *Nicrophorus* species, but found that they did not have an effect (Scott, 1990, Komdeur et al., 2013). These studies were mostly focused on cobreeding, though, so burial times between one and two beetles weren't examined.

Study Sites

Wild *Nicrophorus investigator* were caught using 20 traps spaced 30 paces apart, baited with chicken drumsticks. These traps were set up past the Ore House cabin along the tree line next to the road in Gothic, Colorado. Three additional beetle traps were set up in the Gothic town site, with two by the Gothic Research Center (GRC) and another by the east parking lot.

The captured beetles were kept in plastic containers and fed raw chicken ad libitum. These plastic containers were kept in the GRC, specifically in the bear cage located in the back (See Fig. 4). The experiment also took place within this bear cage.

Determining the effect of Stridulation on Carcass Sequestration in *Nicrophorus investigator* pairs

To determine if stridulation played a role in the movement of the mouse carcass, half of the beetle pairs used had their elytra clipped such that the plectrum (scraper) was removed and the pars stridens (file of teeth) was exposed (see figure 2). This created a V-shaped cut on the elytra and effectively muted the beetles. To determine if cutting the elytra produced a negative effect on carcass transportation, the other half of the beetle pairs used had the outer edges of their elytra cut, such that they weren't muted (see figure 3).

The experiment took place within 10 plastic bins (24 x 17 x 39 cm, Sterilite®) filled with dirt between 7-9 cm. The bins contained a wire screen (25 x 23 cm), which was placed on one side in order to prevent the beetles from digging through the dirt, thus forcing them to travel to the other side of the bin that had exposed dirt. A barrier, made by gluing rocks roughly 1 cm high and between 1-2 cm wide onto a piece of cardboard (24 x 5 cm), was placed along one of the edges of the wire screen to create an obstacle along the beetles' path from one side of the bin to the other (see figure 5).

To begin testing stridulation's role, a previously frozen lab mouse, between 18 and 25 grams, was weighed then placed on the side of the bin that contained the wire screen, followed by a pair of *N. investigator* (one male, one female). The beetles were then enclosed with the mouse for a period of time between 20-30 minutes by being covered with a plastic plant pot (8 x 8 x 9 cm). This was done to introduce the beetle pair to the dead mouse and possibly initiate their transportation of it from one side to the other instead of scurrying off once placed on the mouse. Once the twenty to thirty minutes were up, the pot was removed and the beetles were left alone, notes on their transportation progress (cm) were taken every 30 minutes, from the initiation of the trial, begun around 9 am, until 7:00 pm of that same day. If the beetles did not transport the mouse over the barrier over the course of the day, they were left in the bins overnight and their progress was recorded the following morning before being removed. If the beetles successfully carried the mouse over the barrier, their time was recorded and they were then removed, along with the mouse. 10 different bins (10 trials) were used at any one given time, with a total of 30 different beetle pairs (15 muted, 15 unmuted) used during the course of the experiment. A different mouse was used for each trial.

If the beetles within a specific bin went 1+ hours with no recorded activity, they'd be considered "inactive" and the mouse would be placed back at the starting position. The beetles' positions would then be reset and they'd be enclosed with the mouse for an additional 20-30 minutes before being allowed to continue.

The beetles were marked with a gray Sharpie®. Male beetles were marked on the left side of their pronotum (see figure 2), females had their pronotum's right side

marked (see figure 3). The marks eased the sexing of the beetles during the observations and minimized interference. The beetles' pronota were measured before being placed on the mouse.

Results

N. Investigator pairs were heard stridulating on two distinct occasions: when attempting to mate and when both beetles were attempting to move the mouse over the barrier. On five occasions, the mouse carcass reached the barrier and had both beetles actively working on it, as measured by their attempts to move it over the barrier. Of these five occasions, two were muted trials, meaning that there were three instances where the beetles could have stridulated once at the barrier, and they did on two of those three. They were not heard stridulating when moving the mouse across the screen.

The mean number of minutes the beetle pairs took to move the mouse carcass from the starting point to the barrier was not different between the muted and unmuted pairs. The reset times, which were the amount of times that the beetles and mice had to be placed back in their starting positions due to prolonged inactivity, the number of beetle pairs that successfully moved the mouse to the barrier, and the number of beetle pairs that successfully moved the mouse over the barrier were not different between the muted and unmuted pairs, either (see table 1).

Discussion

This study found that *N. investigator* pairs use stridulation during carcass sequestration when faced with an obstacle, but found no significant difference between the beetles' use of stridulation and lack thereof in terms of their ability to move carrion. Stridulation was not heard when the beetle pairs were moving the mouse across the screen, possibly indicating that they only engage in this behavior when necessary, although we would have to do more trials to see if they engage in this behavior when the obstacle is absent.

One interesting aspect of the experiment was the failure rate for the experiment. Out of the 59 different trials that were carried out, the beetles only managed to carry the mouse carcass to the barrier on 9 occasions. That's a 15.3% success rate, which most likely indicates that the used method of checking carcass sequestration was not the most efficient. It's unknown why this may be, it could be that the beetles didn't think it was worth the effort to move the mouse across the bin, or it could also be that the barrier was too difficult to bypass. The rocks used in the experiment were about 2 cm high, its possible that the energy expenditure to get the mouse over the rocks would have proven too great for the beetles to deem it worthwhile, since they still have to bury the mouse once over the barrier, and then take care of the brood.

One possible solution that could be worked on is changing the barrier to something that remains elevated, but is flatter, unlike the peaks and troughs of the rock

barrier used. One example would be to use a piece of wood 2cm high instead. This would mean that the beetles would only have to get the mouse up to a certain height before they can continue their path.

Other aspects that could be improved upon are the regulation of temperature, since excessive heat or cold could affect the beetles' sequestration efforts. Standardizing the temperatures within the bins, or at least keeping a record of them could be beneficial in rooting out confounding factors that may have led to the low success rate of the experiment. Another one would be to compare the results of this experiment with one that utilizes only one beetle per trial and seeing if beetle pairs have any significant advantage in terms of time for carcass sequestration. It may also be worthwhile to carry out the experiment with beetles whose elytra have not been tampered in any way. These last two suggestions were never implemented due to time constraints and lack of necessary beetles resulting from a later shift in *N. investigator* eclosure. Although the beetles did emerge eventually, I still had difficulty trapping them at my sites. It is known that *N. investigator* has a preference for warm meadows (Smith and Heese, 1995), and my traps were set up along a tree line, surrounded by trees and vegetation, so perhaps a different location for my traps would have improved my chances of trapping beetles.

In conclusion, *N. investigator* pairs were observed using stridulation during carcass sequestration, specifically in the process of moving the carrion over a barrier, but this stridulation didn't seem to have any effect (neither positive, nor negative) on their overall ability to successfully sequester the mouse. The use of stridulation as a means of facilitating cooperation during carcass sequestration has not been studied, with studies regarding burying beetle cooperation usually being on nest-building, feeding young, or brood defense. More studies into this specific use of stridulation in *Nicrophorus investigator* may help further our understanding of how these beetles may possibly communicate and coordinate their efforts during carcass sequestration.

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Tables and Figures

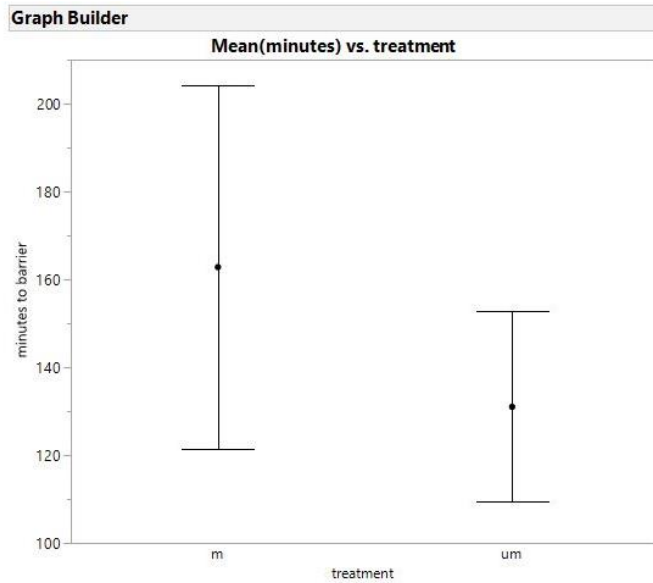


Figure 1: Graph of the mean number of minutes it took for the beetle pairs to move the mouse carcass from the starting point (0 cm) to the barrier (17 cm). Each error bar was constructed using 1 standard error from the mean. $F = 0.1270$ $p\text{-value} = 0.7321$

	Mute	Unmute	χ^2	p-value	significance
Reset times	11/15	9/15	0.603	0.4374	n.s.
Reach Barrier	4/31	5/28	0.279	0.5971	n.s.
Over the Barrier	2/31	3/28	0.345	0.5568	n.s.

Table 1: A table showing the χ^2 values and p-values resulting from the analysis of the times the beetles' positions were reset, the time taken to reach the barrier, and the amount of times it took the beetles to move mouse over the barrier. n.s.= not significant



Figure 2: A picture of a marked and muted male *N. investigator*. The males were marked on the left side of their pronotum with a gray Sharpie® to facilitate sexing. The inner corners of the elytra were cut diagonally in order to remove the plectrum and expose the pars stridens, thus muting the individual.



Figure 3: A picture of a marked and unmuted female *N. investigator*. The females were marked on the right side of their pronotum with a gray Sharpie® to facilitate sexing. The outer corners of the elytra were cut diagonally to mimic the damage done to the muted beetles' elytra.



Figure 4: A map of the site location in Gothic, CO. The red triangle represents the location of the bear cage behind the Gothic Research Center (GRC).



Figure 5: A photo showing the experimental set up in one of the bins.