

***Nicrophorus investigator*: The Effects of Asymmetry in Body Size on the Duration of Bi-parental Care**

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Independent Research and Training in Wildlife Biology Course

Summer 2014

## **Abstract**

*Nicrophorus investigator* is a carrion beetle species commonly found near the Rocky Mountain Biological Laboratory that typically involves biparental care when raising a brood. I observed to see if relative size of the parents influenced the behavior and duration of their stay with their brood. There were four different treatments: two of the same size and two with differing sizes. Although there appeared to not be a significant effect on treatment on duration of male care or larval growth, it was interesting to see that females will tend to their brood no matter the size circumstance.

## **Introduction**

Most species of insects have uniparental or nonparental relationships with their offspring. However, the burying beetle *Nicrophorus investigator* has a biparental relationship with their offspring, with both the male and female parent assisting with rearing the young (Eggert et al., 1998). The rearing consists of locating, burying, and processing a small mouse carcass, as well as carcass maintenance and feeding of offspring (Safryn and Scott, 1999).

The reproductive behaviors in the burying beetle are complex. The adult flies to locate a mouse carcass. Once it finds a carcass, *Nicrophorus* adults will begin to prepare the carcass. If a male arrives first, he will release pheromones to attract a female *Nicrophorus*. The pheromone will attract both male and female *Nicrophorus*, which leads to fights. The respective sex will fight each other which is usually the larger of the competing beetles, will pair with each other and begin to prepare the carcass (Safryn and Scott, 1999).

The pair will first drag the carcass to the preferred location. Then they will remove the fur from the carcass, shape it into a ball, deposit oral and anal gland secretions to preserve the carcass, and bury it. The carcass is buried to hide it from predators and other *Nicrophorus* that will try to compete for the carcass. After the paired beetles have mated, the female will lay her eggs away from the carcass. When the larvae are close to hatching, the parents will then create a depression into the carcass and regurgitate into the depression to soften the food. In the beginning stages of the larval growth, the parents will feed them by regurgitating into their mouths. The larvae begin to feed on their own in about four days, but the parents remain to protect against intruders and maintain the carcass. When the larvae consume the entire carcass they move into the soil, where they will remain until next June (Eggert et al., 1998).

Throughout this three week long process of raising their brood, parents typically stay together. However, Dr. Smith has observed in the field, instances where there is only one parent raising the brood. It is usually a solo female raising them rather than a solo male, and the female beetle is generally larger than the mean size of the population. When pairs are found, they tended to be similar in size. In this experiment I determined how body size, size asymmetry, and sex influences the pattern of biparental versus uniparental relationships. The fitness consequences of uniparental versus biparental care are important to understand the evolution of parental care and the variations observed in natural situations

To test this I designed an experiment pairing similar sized and different sized individuals. If the combination is large female and large male or medium female and medium males, then I predicted that they will remain as a pair (biparental) because both would be eating about the same amount and neither will be able to fight off the other. If they differ in size (large female and medium male, or medium female and large male) then the size difference may allow the larger

individual to evict the smaller of the pair. If there is a solo large female, she may have fought the male out of the nest so that she can raise the brood herself. This may occur if there is a cost to having the male present, for instance, if the male eats too much, leaving less food for the larvae. It is also possible that the male leaves willingly, if he thinks the female is big enough to raise the brood herself, or effectively fight off intruders on her own. The advantage to him of leaving early is to find another carcass, and mate with another female to pass on his genes to more offspring. To distinguish these two, I looked for injuries such as a missing antennae, which indicates a forceful rather than a voluntary leave. For each treatment I measured the duration of male and female care by observing the presence or absence of the male and female with the larvae, or on the surface of the soil. To determine the success of uniparental versus biparental care, I measured the size and number of the brood when they completed their larval growth period.

## **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 was placed on top of the dirt and some water was poured on top to moisten dirt and to help thaw the chicken. 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 (see map of locations). Cans were checked daily and all *N. investigator* were collected and returned to the captive beetle facility where they were watered and fed daily.

The experimental design had four treatments, with 12 replicates per treatment: medium female and large male, medium female and medium male, large female and large male, large female and medium male. Adult beetles (N=96) were sorted by sex, elytra and pronotum width.

An ANOVA of the differences between male and female pronotum mm and elytron mm indicated significant size differences between the asymmetrical pairs, and equal sized (difference = 0) pairs (ANOVA, pronotum mm,  $F = 12.7109$ ,  $p < 0.0001$ ; elytron mm,  $F = 73.520$ ,  $p < 0.0001$ ). Each pair was provided with a dead mouse, Each of the 48 breeding boxes were randomly assigned a size pairing with a frozen 25-26 gram laboratory mouse left to thaw. The size of the mouse carcass was kept constant (ANOVA,  $F = 1.8679$ ,  $p = 0.149$ ), to not affect the resource quantity and therefore influence the potential total brood mass. After a five hours of thawing, each pair was placed in a breeding box. The breeding box is a clear plastic box (7 by 5 by 13.5m), with a ventilated lid. The breeding boxes were then place outdoors in a carnivore-proof cage.

Observations of the following were made daily: carcass preparation stage, time and day of burial, egg laying, larval hatching, male or female surface activity (away from carcass/brood ball with larvae or attempts to crawl out), and injuries to adults. When the larvae showed signs of development completion, meaning they finished consuming the mouse carcass and there were no more larvae directly near the carcass, I weighed and measured each larvae.

In the analyses, I compared the number of larvae, brood mass, and mean size of the brood to the four treatments. I also compared the number of days the male stayed within close proximity of the carcass for each of the four treatments. If there were two consecutive days of the male not showing signs of being near or with the brood, the days prior were counted as the day the male “left” the brood chamber. To analyze these results, I used a one way ANOVA and then used the Tukey-Kramer test to determine which means were significantly different (JMP 11).

## Results

The treatment type did not have a significant effect on the number of days the male stayed with the brood as shown in Figure 1. (ANOVA:  $F = 0.4197$ ,  $p = 0.7398$ ). The number of larvae (ANOVA,  $F = 0.0749$ ,  $p = 0.9731$ ) (Figure 2), mean mass of larvae in each brood (ANOVA,  $F = 2.5803$ ,  $p = 0.0658$ ) (Figure 3) and total brood mass (ANOVA,  $F = 0.4902$ ,  $p = 0.6909$ ) (Figure 4) were also not significantly affected by the treatment type. For the marginally significant effect of treatment on number of larvae in a brood, a post-hoc test (Student's  $t$ ) was conducted to detect differences among treatments. This test indicated that larvae reared in MM parental combinations were significantly larger than those reared by the LL and LM combination ( $p = 0.0241$  and  $p = 0.0295$ , respectively). Also, larvae reared in the ML combination were marginally larger than the LL ( $p = 0.1444$ ).

The parental combination did not influence the time of burial to the first larvae (ANOVA,  $F = 0.407$ ,  $p = 0.7042$ ). In contrast, the duration of larval development from the time they hatched to the time they finished was effected by parental size (ANOVA,  $F = 3.7377$ ,  $p = 0.0179$ ). Post hoc test indicated that the medium female and large male took significantly longer to develop than the other treatments.

## Discussion

The purpose of this study was to see if relative size of paired parents influenced the duration of parental stay, with the prediction that larger females would be more likely to parent alone (uniparental), and similar sized pairs would remain together to rear the brood for a longer period (biparental) My results indicate that there is no effect of parental size asymmetry on the duration of male care. Because each treatment was essentially the same in terms of the duration

of male and female parental care, it is not surprising that there were also no differences among the treatments on larval development (size, number, total brood mass). These results were surprising because they are in contrast to a previous study which found that males paired with larger females had a shorter duration stay in the brood chamber (Manvir Singh, 2011).

My study differed from Singh's study in terms of using another size treatment (Medium female and Large male). In my results, Medium-Large pairs took longer than Large-Medium. Same sized pairs (Large-Large and Medium-Medium) took longer than different sized pairs (Medium-Large and Large-Medium) indicating that large females were faster than medium sized females when burying the carcass. Overall, Large-Large=Large-Medium=Medium-Medium from the first signs of larvae until the larvae completed development.

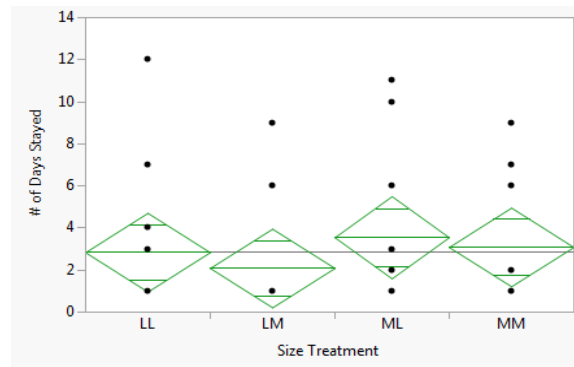
In Manvir Singh's study, he used coffee cans instead of the breeding boxes used in my study. It is possible that the shallower breeding space may have affected the behavior of the adults, especially the males, and my ability to observe/detect "leaving" because it was difficult to distinguish their proximity to the brood ball and chamber. For future replication of this study, I would recommend using deeper or longer boxes to better detect the proximity to the carcass (more opportunity for "away" and "leaving" behaviors. Identifying the sex of the adult beetles was difficult and often disturbed the carcass or the beetles themselves. I would also recommend marking the sexes. Finally, on some observations I was not able to readily locate the two parents, so could not record their locations. Some mechanism to detect and record them at every observation period would also be better.

The role of parents in the rearing of burying beetle larvae includes carcass location, burial, preparation with anti-microbial secretions, and protection from intruders. Clearly, uni-

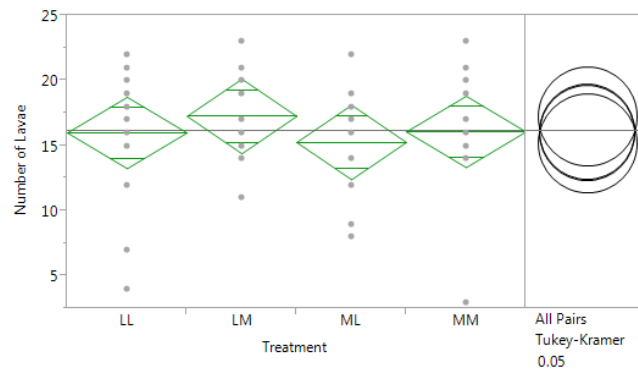
parental care is possible (by females) for all of these behaviors. The role of males, and therefore the evolution of biparental care, is of considerable interest. Why do males assist at all and remain in the proximity of the brood? In this experiment, all the males stayed the same amount of time, and the larvae developed similarly. Because the adults fight for carcasses, it is possible that larger size has the advantage at the very beginning of the reproductive bout; and for the exclusion of intruders. Because there were no conspecific intruders in this experiment, perhaps the cue to remain or leave for males was missing. This could be addressed by designing an experiment with the same treatments, but with the addition of conspecific intruders.

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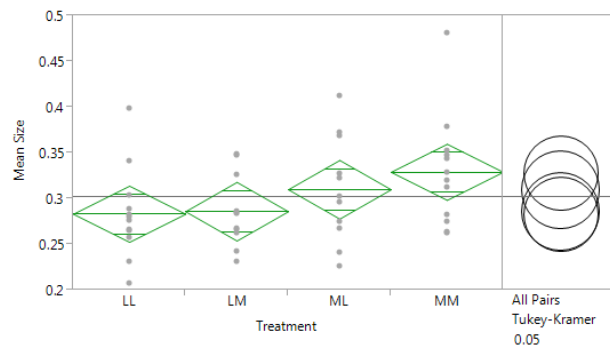
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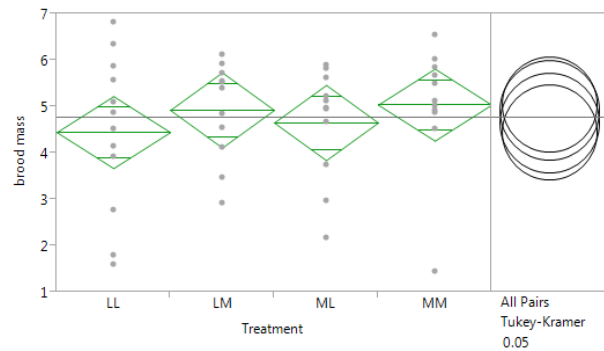
**Figure 1.** Number of days of male care after the first sign of eggs.



**Figure 2.** Number of larvae based on the type of treatment.



**Figure 3.** Comparison between mean sizes of broods from the treatments.



**Figure 4.** Mass of the brood from each treatment.

