

Effects of parental care on brood success and quality
in the burying beetle *Nicrophorus investigator*

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Abstract

Across all species, biparental care is an atypical and intriguing adaptation. Burying beetles, (Coleoptera: Silphidae) exhibit a large range of biparental care to offspring, involving the upkeep of their sole food source, direct feeding to larvae, and protection of the brood from a wide variety of intruders. However, as previous studies have found, in some cases the presence of two parents in the brood chamber is actually detrimental to the brood overall. In this experiment, we observed the success and quality of *Nicrophorus investigator* broods raised with varying degrees of parental care in the hopes of determining a driver for the persistence of this biparental care behavior in the species. Contrary to the expected, it was observed that *Nicrophorus investigator* larvae are able to develop normally without parental care post-hatching, uniparental care is the most successful type of parental care for brood quality under laboratory conditions, and single males are equally good parents as single females.

Introduction

The evolution, costs, and benefits of parental care are long and widely disputed [1,2,3]. This adaptation may come in many forms, broadly including biparental and uniparental maternal and paternal care [4]. Most species do not offer any care to offspring, while others have developed elaborate ways of helping out their young [6]. These parenting tactics each have their own benefits and repercussions.

The largest trade-off classically suggested with any type of parental care is between offspring survival and future reproductive success [7]. While parents caring for a brood will typically see healthier and more successful offspring, opportunities to increase their own fitness are cut shorter as the time they spend with their current brood increases. So what necessitates parental care? What causes parents to stay with their current offspring rather than seek out additional reproductive opportunities and, furthermore, what factors necessitate care from two parents rather than one?

Our study species, *Nicrophorus investigator* provides elaborate biparental care to offspring. *Nicrophorus* beetles raise their young on small vertebrate carcasses, which they must win from competing beetles of the same or different sex and species [6-9]. In addition, they must contend with various organisms that also dwell on or consume carrion,

such as flies and ants [7,11]. Sometimes, multiple pairs or a single individual beetle will bury the attained carcass, but usually a single pair will bury their prize cooperatively [12]. Once buried, a female will lay eggs in the soil surrounding the carcass and soon after hatching, larvae will crawl to the brood chamber created by the parents [6]. The parents will hydrate and prevent the carcass from rotting through anal and oral secretions, regurgitate carrion to the larvae, and protect the brood from intruders and usurpation [6-9,12-16].

With such a seamless operation, it would appear that the biparental care exhibited by burying beetles could be nothing but beneficial to offspring. However, while larvae benefit from parental care throughout development [15] it appears that a male presence in addition to a female in the brood chamber offers no significant benefits to brood success or quality [15, 17]. As such, the factors influencing the evolution of biparental care in these beetles is unclear.

In an attempt to clarify some of the benefits of biparental care by burying beetles, we examined one leading hypothesis in *Nicrophorus investigator*: due to the hydrating and anti-microbial substances secreted by parents to prevent carrion from rotting, upkeep of a carcass and direct larval care necessitates the cooperation of two parents. This may be particularly true for large carcasses, which require more preparation and harbor more larvae. Thus, we predict that broods raised by two parents throughout larval development will have the most success, particularly on large carcasses, followed by those given uniparental care. We do not expect successful broods on carcasses without any parents.

Methods

Beetle Collection and Storage

At the beginning of the carrion beetle breeding season (late June), twenty beetle traps were constructed and set (see attached map for location). These traps consisted of metal cans filled three-quarters full with soil from the surrounding area and suspended in the air from predators by wiring to a tree, post, or similar structure. All traps were baited with frozen mice caught from the Gothic town site. Typically, two small mice (less than or equal to sixteen grams) were placed, still frozen, in the trap, which was then covered with a wire mesh lid with a quarter-sized hole in the center. This lid was secured to the trap with

a rubber band and also wired onto the can to prevent robbing by predators. All traps were watered and checked roughly every two days unless rain naturally maintained moist soil in the can. *Nicrophorus* beetles were collected from cans, but all other organisms caught in the traps were ignored. Bait was replaced as needed.

Beetles caught from the wild traps and collected from Rosemary Smith's Rocky Mountain Biological Laboratory overwinter pits were stored in plastic boxes lined with moist paper towels and were separated by beetle type (wild-caught vs. overwintered), species, and sex. These boxes were cleaned and the beetles fed raw chicken every few days when necessary. Boxes were stored in an outdoor, bear-proof cage receiving both sun and shade.

Experiment Setup

Soil for all experiments was taken from the town site and sifted into a large storage container to remove all large rocks and extraneous materials. For the first experiment, eighty plastic containers were filled with this sifted soil. The containers were covered by fitted plastic lids with a circle of air holes in the center.

One female was added to each box. All females used were those that emerged from the overwinter pits between four and ten days before the start of the experiment. The pronotum of each female was measured, and only females with pronota between 5.0 and 6.0 millimeters were used. After each box housed one female, one male was added, as well. These individuals were also those that had emerged four to ten days earlier from the overwinter pits and were measured to have pronota between 5.5 and 6.5 millimeters.

White laboratory mice arrived on ice and were immediately weighed out on an electric balance and separated into weight classes. Using a random number generator (numbers 1 through 8), the treatment of each box was determined: no parental care, male care only, female care only, or care from both parents, each on either a small or large carcass. Treatments 1 through 4 received "small" mice (17.5 – 19.0g), while treatments 5 through 8 were given "large" mice (29.0 – 34.0g). One appropriately sized mouse was added, still frozen, to each container within two hours of the addition of the beetle pair. Mice were laid flat in the center of each container, which were immediately closed. This experiment was also stored in the outdoor cage.

Data Collection

For five days following the introduction of the beetles to their breeding boxes and carcasses, boxes were checked twice daily for burial status and appearance of eggs. This monitoring was strictly visual, with observations being made through the sides of the container and the occasional removing of a container's lid when necessary. During this period, neither beetles nor carcasses were touched. Burial status was defined as a carcass on the surface, one-quarter buried, half buried, three-quarters buried, or fully buried. Eggs visible through the sides or bottom of the container were counted. In two boxes, carcasses were not moved within three days of starting the experiment. The pairs in these boxes were replaced with new individuals fitting the same criteria used for all other pairs.

The fifth day following introduction, all boxes were checked for larvae in both the morning and afternoon. After finding no larvae in the morning, all boxes were moved to a warmer area for the entire day. Throughout the rest of the experiment, all boxes were moved to this area in the morning (experiencing average high and low temperatures of 35.5 and 7.8 degrees centigrade) and back to the outdoor cage in the evening (average high temperature of 21.5, average low of 5.6 degrees centigrade). To check boxes for larvae, the process was kept as non-invasive as possible. Some larvae were clearly present on the surface of the carcass, while other times the carcass needed to be fully removed from the box to determine whether larvae had emerged or not. After checking for larvae, the carcass would be replaced as closely to how the beetles had arranged as was replicable.

Depending on which treatment the box was assigned, each observed to have larvae was either left alone or modified. Based on treatment, the male, female, or both beetles would be removed. When a male was removed, it was immediately put in a 1.5mL centrifuge tube. After checking all boxes, a rack filled with the removed males in their tubes was put in a freezer. At the conclusion of this experiment, ethanol was added to the tubes, which were placed again in the freezer for storage. When a female was removed, it was marked with a pin on its wing case for identification for a subsequent experiment. ID was based on treatment and order of removal, and corresponded with a number 1 through 10. The wing case was divided into four quadrants by the distinctive *Nicrophorus* pattern with a maximum of three pinholes in one quadrant based on ID number. As the highest ID number possible was ten, four holes in one individual's wing case was the maximum.

According to treatment, the female was then added to a breeding box with other females from the experiment. These boxes contained no soil and were lined with moist paper towels. The boxes were cleaned and the beetles fed raw chicken every few days when necessary.

Boxes were checked for larvae twice daily for three days. For four days following this period, boxes were checked only once daily for the appearance of larvae. Only the boxes that had not yet been observed to contain larvae were checked during this process.

After larvae left the carcass and began to move about the soil inside the box, they were removed, counted, and weighed. A maximum of four larvae were taken from each brood and suspended in ethanol in microcentrifuge tubes for future paternity tests. Any excess of larvae were moved to the Rocky Mountain Biological Laboratory overwinter pits. Carcasses were discarded and the soil and parents remaining were removed. Females were used again in a subsequent experiment examining the success of their second broods, while all males were frozen in centrifuge tubes to which ethanol was later added for future genetic analyses.

Data Analysis

To assess the probability of success across broods with different types of parental care and soil conditions, we used a contingency table in the program JMP. Similarly, we also used a one-way analysis of variance in JMP to investigate the variance in total weight produced, number of larvae produced per gram of carcass, and efficiency across broods with varying degrees of parental care. Because some parents died before the conclusion of the experiment and the muddiness of the surrounding soil proved significant in decreasing the success rate of a brood, only families where the imposed treatment was carried out until the end of the experiment and the soil was dry were used in data analysis.

Results

Overall, brood success was 58.8 percent, measured as the number of broods with at least one larva completing development from those observed to have larvae emerge. The eight treatments imposed upon broods did not differ in their success, as shown in Figure 1 ($\Lambda=0.2117$). The muddiness of the surrounding soil, however, had a negative effect on

success ($\Lambda=0.0086$), so these boxes were excluded from all analyses, regardless of treatment. Surprisingly, the number of maggots present in the soil and on the carcass did not have an effect on brood success ($\Lambda=0.7544$).

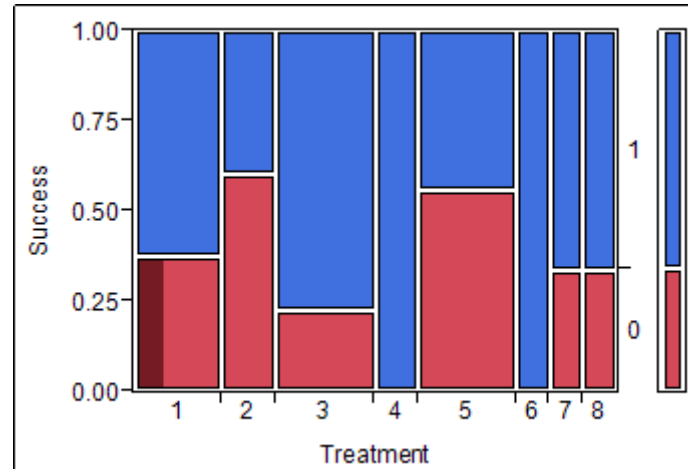


Figure 1. The effect of parental care and carcass size on the success rate of a brood, where the value 1 denotes a success and 0 is a failure.

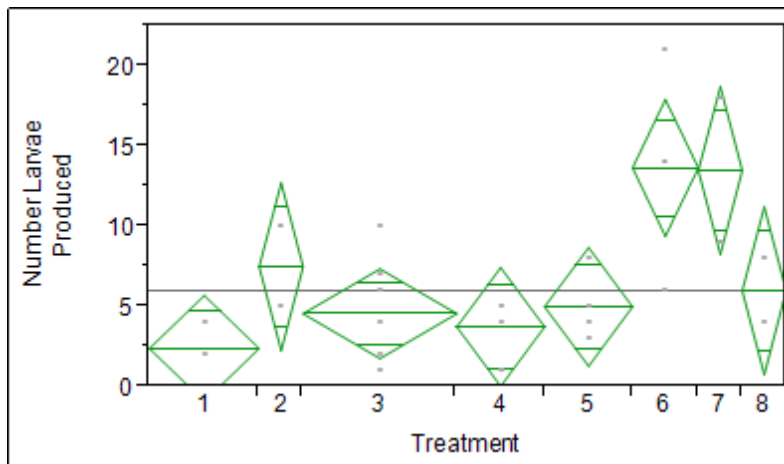


Figure 2.1 The number of larvae produced by broods with varying parental care on different-sized carcasses

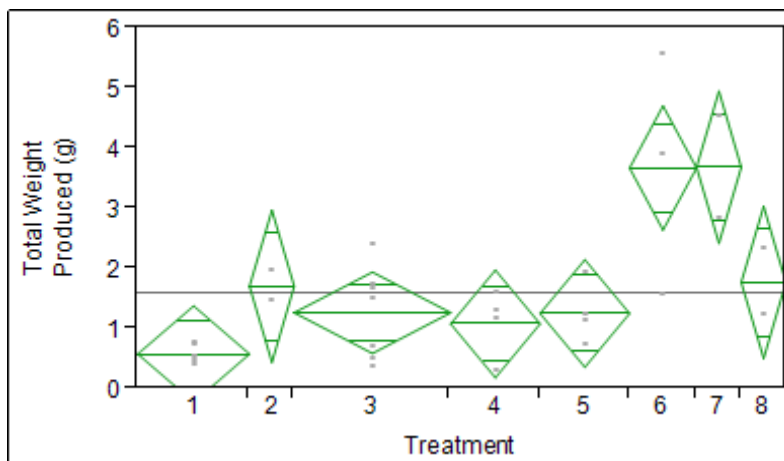


Figure 2.2 The total weight produced by broods with varying parental care on different-sized carcasses

As shown in Figures 2.1 and 2.2, treatment did have an effect on the number of larvae produced ($p=0.0034$) and total brood mass ($p=0.0009$), with broods raised uniparentally on large carcasses having the most success on both accounts. However, because of the low number of replicates for each treatment and the direct relationship found between total brood mass and carcass mass ($p=0.0047$), a variable that allowed for comparison of brood success across the two carcass size categories was calculated. The first was brood growth efficiency, reflecting the grams of larvae reared from the grams of mouse carcass available. The second variable is the number of larvae per gram of mouse, which is also a measure of parental fitness that controls for the resource quality.

Using these variables, we found that the type of parental care provided to broods did have a significant effect on brood quality overall. Broadly, any type of parental care was shown to increase the efficiency of a brood (Figure 3.1) as well as the number of larvae produced per gram of carcass (Figure 3.2).

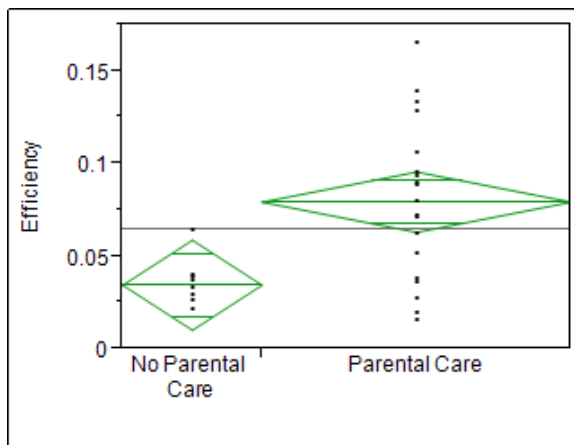


Figure 3.1 The efficiency of broods with and without parental care on both large and small carcasses ($p=0.004$)

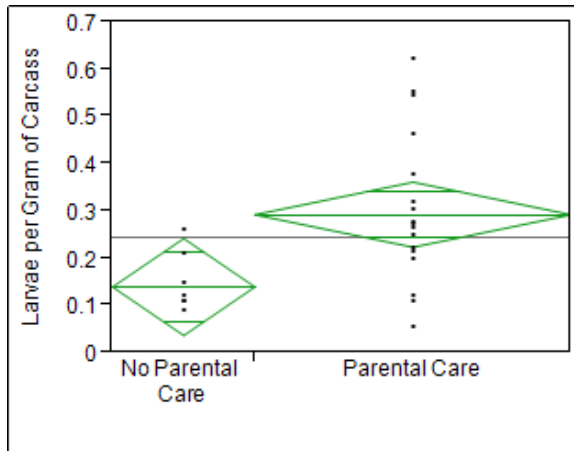
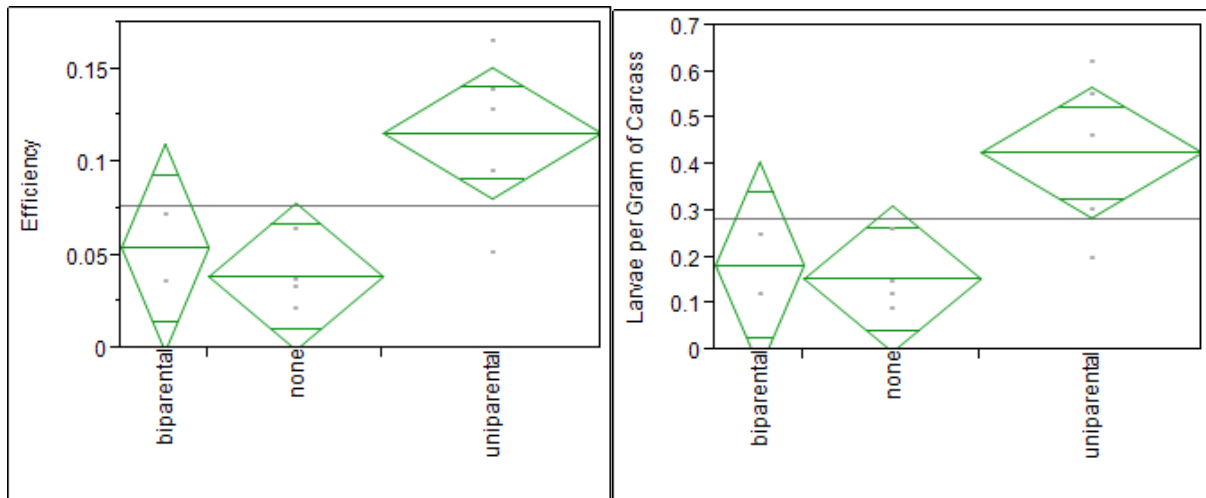
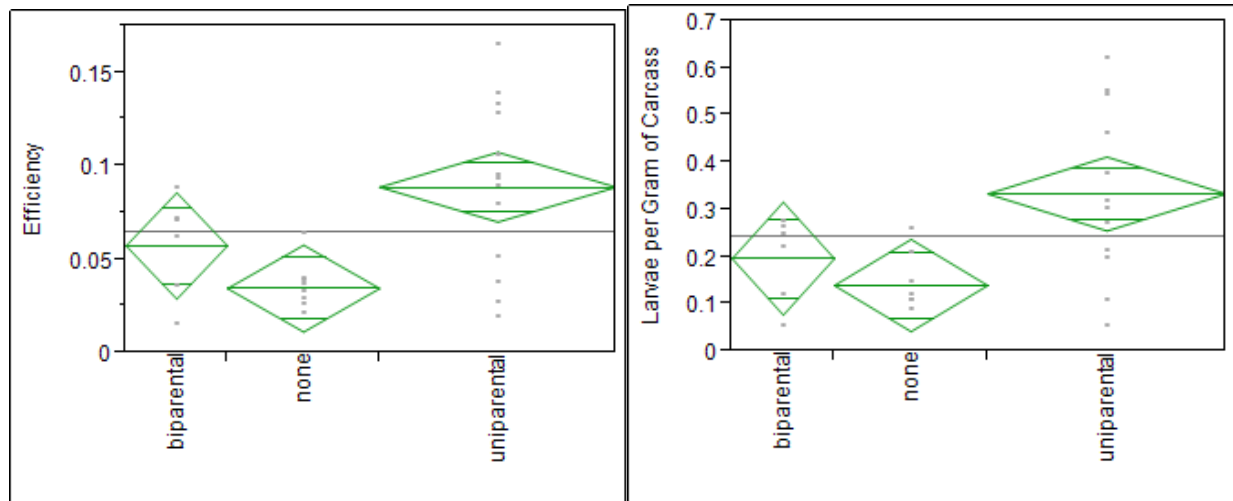


Figure 3.2 The number of larvae produced for each gram of carcass present between broods with and without parental care on both large and small carcasses ($p=0.0171$)

Contrary to our hypothesis, it was observed that within parental care, uniparental care actually produced the most quality broods with the highest efficiency (Figure 4.1, $p=0.0241$) and number of larvae for each gram of mouse (Figure 4.2, $p=0.0379$) on large carcasses. Furthermore, this result was replicated across all carcass sizes (Figures 4.3 and 4.4, $p=0.0032$ and $p=0.0101$ respectively). For both these variables, broods lacking parental care fared the worst, while biparental care was somewhere between no and uniparental care, but did not vary significantly from either treatment.



Figures 4.1 and 4.2 The efficiency and number of larvae per gram of carcass produced for broods with no, uniparental, and biparental care on large carcasses only



Figures 4.3 and 4.4 The efficiency and number of larvae produced per gram of carcass for broods with no, uniparental, and biparental care across all carcass sizes

Further, uniparental care by males produced broods with the highest number of larvae per gram of carcass (Figure 5.1, $p=0.0078$). However, with regard to efficiency, female only uniparental care was just as efficient as male only, which altogether were significantly more efficient than broods with no parental care (Figure 5.2, $p=0.0040$). In both cases, biparental care again fell in the middle of all treatments and was not significantly more efficient and did not produce a significantly higher number of larvae per gram of mouse carcass than broods lacking parental care.

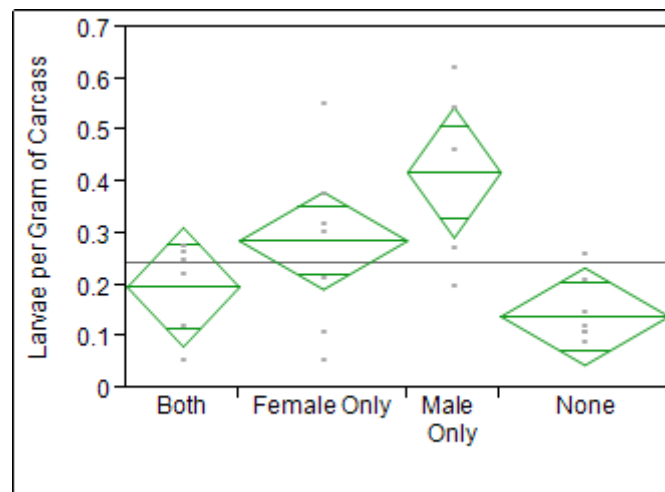


Figure 5.1 The number of larvae per gram of carcass across broods with no care, male-only, female-only, and biparental care on both large and small carcasses

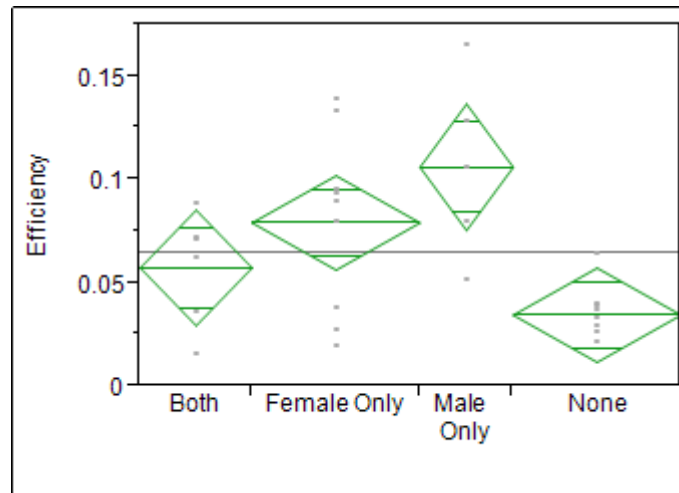


Figure 5.2 The efficiency of broods with no care, male-only, female-only, and biparental care on both large and small carcasses

Discussion

The results of this experiment indicate that like *N. defodiens* and *N. tomentosus* [18], *N. investigator* larvae are able to develop normally in the absence of parental care post-hatching. This is an interesting result, as it contrasts with our observations that *N. investigator* adults are commonly found attending larvae in the field, and readily assume parental care behavior under laboratory conditions. In addition, uniparental care was found to be the most favorable for brood success and that males are perfectly capable of raising young on their own in *Nicrophorus investigator*. These findings are consistent with those reported by Pukowski (1933) and Trumbo (1992), who noted variation in the amount of parental care required across the *Nicrophorus* genus: *N. orbicollis* and *N. sayi* do not develop correctly in the absence of parental care whereas *N. defodiens* and *N. tomentosus* are able to develop normally. Based on these conclusions, it is reasonable to believe that two parents providing care under these conditions is excessive, and the costs of two parents outweighs the benefits of their care. These costs are unknown, and may be anything from adult carrion consumption to conflicts between parents and/or larvae. However, in a natural setting, biparental care may be most effective with one parent, male or female, caring for the brood and the other protecting it from intruders and usurpation.

The relatively higher success of uniparental care over biparental care suggests that two parents providing care under this controlled condition is unnecessary, even on a larger

carcass. In addition, the results suggest a cost of two parents that outweighs the benefits of their dual care. These costs likely include carrion consumption by the adults and conflicts between parents. Numerous studies have supported the benefits of having two adults present in situations where intruders, especially congenics or conspecifics, are likely [6,16-18]. Many of these studies have shown that females tend to spend more time caring for the carcass and brood, while males spend more protecting the carcass and brood from intruders and usurpation, often leaving the brood chamber before the completion of larval development and several days before the female departs [19-22].

Further experiments that examine the roles of adults present in the brood chamber under more natural conditions are warranted. In particular, the role of adults in defending against intruders and competitors such as other burying beetles and many species of fly and ant [6-9, 11] should be examined.

Finally, the more delayed effects of parental care need more investigation. The costs of brood rearing may be substantial, thereby influencing the resources available in adults, male or female, for future reproduction. Assistance in brood care may reduce these costs, enhancing body condition and increasing the success of second broods. Thus, male assistance may be of benefit to females. However, for the male to benefit, he would need to share in the enhanced reproductive success of the female in her second brood. As beetles can store sperm [23], the paternity of a subsequent brood based on amount of time spent by a male with a female's first may be important. Genetic analysis of the broods and adults collected in this experiment will aid in investigation.

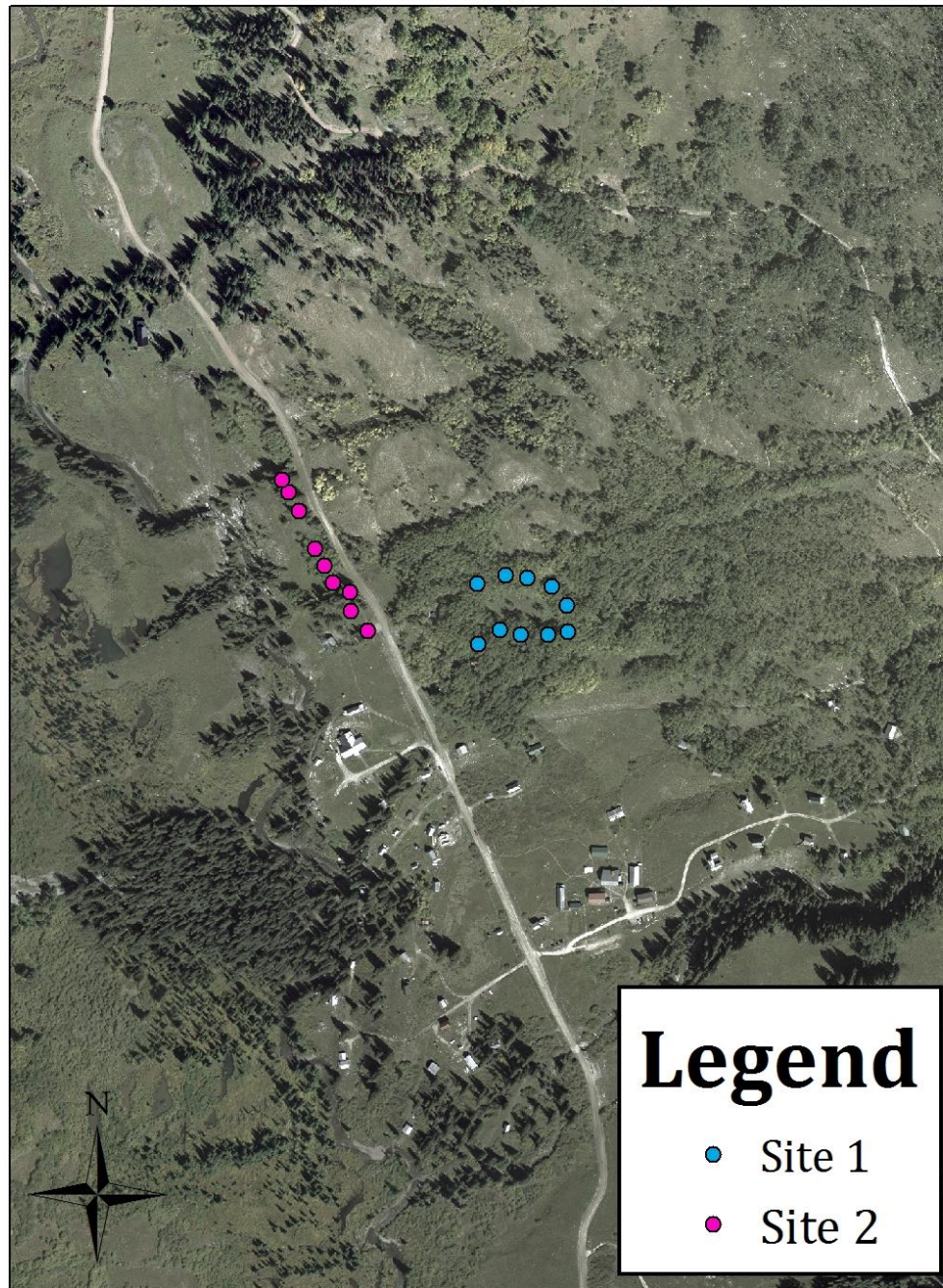
In a genus with high competition and widely spread individuals, many aspects of life history and reproductive methods may push for biparental care of broods. While we cannot assume that in every case uniparental care is actually more effective than the biparental care observed in these individuals, we can conclude from our data that upkeep of a carcass and direct feeding to larvae are not major causes for this rare and intriguing behavior.

Works Cited

1. M. R. Gross. "The evolution of parental care". *The Quarterly Review of Biology*, vol. 80, no. 1, pp. 37-45, 2005.
2. A. G. Zink. "Quantifying the costs and benefits of parental care in female treehoppers". *Behavioural Ecology*, vol. 14, no. 5, pp. 687-693, 2002.
3. J. D. J Gilbert, L. K. Thomas, and A. Manica. "Quantifying the benefits and costs of parental care in assassin bugs". *Ecological Entomology*, vol. 35, pp. 639-651, 2010.
4. P. T. Smiseth. "What is parental care"? Oxford University Press, 2012.
5. J. D Gilbert and A. Manica. "Parental care trade-offs and life-history relationships in insects". *American Naturalist*, vol. 176, no. 2, pp. 212-216, 2010.
6. E. Pukowski. "Ökologische untersuchungen an *Necrophorus F*". *Zeitschrift für Morphologie und Ökologie der Tiere*, vol. 27, no. 3, pp. 518-586, 1933.
7. D. S. Wilson and J. Fudge. "Burying beetles: intraspecific interactions and reproductive success in the field". *Ecological Entomology*, vol. 9, no. 2, pp. 195-203, 1984.
8. D. S. Wilson, W. G. Knollenberg, and J. Fudge. "Species packing and temperature dependent competition among burying beetles (*Silphidae*, *Nicrophorus*)". *Ecological Entomology*, vol. 9, no. 2, pp. 205-216, 1984.
9. S. T. Trumbo. "Interference competition among burying beetles (*Silphidae*, *Nicrophorus*)". *Ecological Entomology*, vol. 15, no. 3, pp. 347-355, 1990.
10. M. P. Scott and J. F. A Traniello. "Behavioral cues trigger ovarian development of the burying beetle *Nicrophorus tomentosus*". *Journal of Insect Physiology*, vol. 33, pp. 693-696, 1987.
11. M. P. Scott. "Communal breeding in burying beetles". *American scientist*, vol. 84, no. 4, pp. 376-382. 1996.
12. W. W. Hoback, A. A. Bishop, J. Kroemer, J. Scalzitti, and J. J. Shaffer. "Differences among antimicrobial properties of carrion beetle secretions reflect phylogeny and ecology". *Journal of Chemical Ecology*, vol. 30, no. 4, pp. 719-729, 2004
13. R. L. Rana, W. Wyatt Hoback, N. A. A. Rahim, J. Bedick, and D. W. Stanley. "Pre-oral digestion: a phospholipase A₂ associated with oral secretions in adult burying beetles, *Nicrophorus marginatus*". *Comparative Biochemistry and Physiology B*, vol. 118, no. 2, pp. 375-380, 1997.
14. S. Suzuki. "Suppression of fungal development on carcasses by the burying beetle *Nicrophorus quadripunctatus* (Coleoptera: *Silphidae*)". *Entomological Science*, vol. 4, no. 4, pp. 403-405, 2001.
15. J. K. Müller, A. K. Eggert, and S. K. Sakaluk. "Carcass maintenance and biparental brood care in burying beetles: are males redundant?" *Ecological Entomology*, vol. 23, no. 2, pp. 195-200, 1998.
16. I. C. Robertson. "Nest intrusions, infanticide, and parental care in the burying beetle, *Nicrophorus orbicollis* (Coleoptera: *Silphidae*)". *Journal of Zoology*, vol. 231, pp. 583-593, 1993.
17. S. T. Trumbo. "Interspecific competition, brood parasitism, and the evolution of biparental cooperation in burying beetles". *Oikos*, vol. 69, no. 2, pp. 241-249, 1994.

18. M. P. Scott. "Brood guarding and the evolution of male parental care in burying beetles". Behavioral Ecology and Sociobiology, vol. 26, no. 1, pp. 31-39, 1990.
19. S. T. Trumbo. "Monogamy to communal breeding: exploitation of a broad resource base by burying beetles (*Nicrophorus*)". Ecological Entomology, vol. 14, pp. 289-298, 1992.
20. I. A. Fetherston, M. P. Scott, and J. F. A Traniello. "Parental care in burying beetles: the organization of male and female brood-care behavior". Ethology, vol. 85, no. 3, pp. 177-190, 1990.
21. M. P. Scott and J. F. A Traniello. "Behavioural and ecological correlates of male and female parental care and reproductive success in burying beetles (*Nicrophorus spp.*)". Animal Behaviour, vol. 39, no. 2, pp. 274-283, 1990.
22. M. P. Scott and D. S. Gladstein. "Calculating males? An empirical and theoretical examination of the duration of paternal care in burying beetles". Evolutionary Ecology, vol. 7, no. 4, pp. 362-378, 1993.
23. J. K. Müller and A. K. Eggert. "Paternity assurance by "helpful" males: adaptations to sperm competition in burying beetles". Behavioral Ecology and Sociobiology, vol. 24, no. 4, pp. 245-249. 1989.

Beetle Trapping Sites



A map depicting the location of our beetle traps in both sites near the Gothic, Colorado town site.

