

**Seasonal reproductive potential and
iteroparity of the burying beetle
(Coleoptera: Silphidae)**

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Abstract: Iteroparity of the burying beetles of the western montane regions of the United States was investigated. Rodent carcass resources were offered to track reproduction and allow for marking of breeders during the first of two rounds of observations. Second round observations were limited to looking for repeat breeders. No breeders from the first round returned and bred during the second round. *Nicrophorus investigator*, *N. defodiens* and *N. guttula* occur at the study sites. However, only *N. investigator* and *N. defodiens* were found. *N. defodiens* was excluded from analysis as none were successful in reproducing during the experiment.

1. Introduction

Understanding reproductive behaviors and potentials of any living organism is an essential aspect to ecology. It is in the production of viable offspring we find a species' fitness, which serves as a means of survival of the genetic material passed on from generation to generation. Males are typically able to increase their fitness by mating with multiple females; while females are able to maximize their reproductive success by maximizing the number of viable eggs they produce (Arnqvist & Nilsson 2000). While the number of sexual encounters may be an integral part of the reproductive behaviors of most living organisms, often in nature there exists a precisely-timed seasonal cycle of reproduction. These cycles are not only fundamental to animal populations adapting to northern environments but also to those living at high, montane altitudes (Aleksiuk & Gregory 1974).

Iteroparity indicates multiple broods produced by an organism each year or season. Variation among reproductive behaviors can be seen across the Arthropoda phylum with several factors influencing whether or not a species is iteroparous or semelparous. Studies conducted in laboratory experiments suggest burying beetles are iteroparous but that the number of reproductive attempts by the beetles is quite small (Nagano & Suzuki 2007). Whether or not this is true in the field is questioned; no studies have been conducted to evaluate iteroparity among burying beetles of the western montane regions of the United States. Of the three species of burying beetles located in the western montane regions, reproductive behaviors are seasonal with slight variation between months during which reproductive periods occur.

Burying beetles (Silphidae: Nicrophorus) are known to locate and bury small vertebrate carcasses, which they then form into brood balls as provisions for their young; both male and female burying beetles remain with their offspring until the carcass is consumed (Trumbo 1990). Factors influencing burying beetles' reproductive activities may include variation in emergence times among species as well as sexual maturity rates (Scott 1998). However, little is known about *Nicrophorus investigator*'s and *N. defodiens*' potential for iteroparous behaviors in the montane regions of the western United States.

Additionally, seasonality of the high elevations of the western montane regions has been shown to be influenced by climate change differently than lower elevations (Earthwatch Institute 2000, Inouye et al. 2000). At higher elevations, there is a shorter growing season delimited by a combination of snowpack and temperature which influence reproductive seasonality of burying beetles in the region. In other locations, reproductive seasons of the beetles are longer and much less impacted by the strong winter temperatures and precipitation that begin earlier at higher elevations (Inouye & Wielgolaski 2003). The capacity of burying beetles to produce two or broods during one season is probable given the physiology of the beetles. Whether or not the beetles are actually reproducing two or more times given their short window of opportunity during the summer breeding season is the question this study addressed.

This study hypothesizes that *N. investigator* and *N. defodiens* will produce multiple broods during a single season of reproductive activity as well as display a distinct variance between species. Furthermore, the reproductive potential for iteroparous behaviors among burying beetles was evaluated.

2. Methods and materials

Study organisms. Both *N. investigator* and *N. defodiens* occur at the study sites. Much less is known about the behavior of *N. defodiens* at these sites since its population density is much smaller than that of *N. investigator*. A third species, *N. guttula*, also occurs at the study sites but is rare and was not found during the entire length of the experiment. The adult beetles are diurnal and reproduce only during the summer months. After a male locates a carcass, he attracts a female to it by way of pheromones. They then begin the act of burial and preparation of the carcass, mating several times during the burial process (Smith et al. 2000). Once a carcass is prepared in the chamber, eggs are scattered in the soil around the carrion where larvae develop on the corpse through three instars, or developmental stages. The larvae not only feed on the carrion themselves but are also fed regurgitated carrion from their parents. During the third instar, larvae move away from the carrion and overwinter and pupate in individual chambers in the surrounding soil (Bartlett & Ashworth 1988, Smith et al. 2000). Parents remain with their broods for approximately two weeks, dependent upon developmental stage and time with the carcass (Scott 1998, Scott & Traniello 1990). Considering this time frame against the entire reproductive season, there is a great chance that reproduction may occur more than once during the season, with multiple broods being produced.

Study Sites. Study sites were located within the vicinity of the Rocky Mountain Biological Laboratory (RMBL), located in the Upper East River Valley, Gothic, Gunnison County, Colorado, USA (Latitude 38° 58', sp. Longitude 107° 00'; 2900m elevation). Previous field studies on *Nicrophorus* have been conducted at the first of two selected sites: (1) Research Meadow: an open and steeply sloping (15-35°) meadow located in the RMBL area and (2) Barr Meadow: an open and less steeply sloping (10-20°) meadow located on the northern end of RMBL in Gunnison National Forest, along the East River.

Materials and Supplies. One bait line was set up at each of the two study sites. At 25 m intervals, a trap was placed along the line (30 traps at the Research Meadow site and 20 at the Barr Meadow site). Each trap consisted of a metal can approximately 18 cm deep and 15 cm in diameter, pierced to allow drainage and filled $\frac{3}{4}$ full with soil, covered with a metal fencing grid to protect from predators. Each grid was reinforced with either

metal stakes in the ground surrounding the metal can or large rocks to hold the grid down.

Each of the traps was baited with a rodent carcass taken from RMBL's 2008 rodent inventory. All rodents were native and wild-caught, trapped on RMBL property. *Peromyscus maniculatus* was used in approximately 95% of the cans; with *Microtus montanus*, *Zapus princeps* and *Tamias* spp. making up the remaining percentage of rodents used. Since *Nicrophorus* spp. generally only bury and reproduce on carcasses weighing between 16 and 48 grams (Smith and Heese 1995, Smith & Merrick 2001) and only approximately 60 rodents from RMBL's 2008 rodent inventory fell within this range, the remaining cans were baited with two rodents tied together with string. Only 29 cans were baited during the first round at the Research Meadow site. Also, during the second round of baiting at the Barr Meadow site, only 18 cans were given carcasses as there were not enough for all 20 cans. Dead grass and leaves were collected and placed underneath rodents to allow for shade from the sun once beetles buried them. This prevented the soil from drying out, allowing moisture to be retained.

Beetles were allowed to bury the rodent and complete brood care. When the broods completed development, the adults departed as they would naturally. Broods were collected two weeks after burial (when they completed development and the adults had left). Then, the cans were re-baited once more with a fresh carcass.

Beetle Visitation and Reproduction. Carcasses were checked once per day in the two days after I placed them outside. They were then checked approximately five days after being placed outside to check for nesters. I captured and marked all adult beetles that arrived at a carcass during the first round of baiting at the Research Meadow site with two triangular cuts on the elytra (Smith et al. 2000). In the remaining rounds of baiting, only nesting adults were marked; adult beetles that were successful in winning and burying a carcass were marked with two tiny pin pricks on the surface of their right elytra to distinguish them from those that did not successfully nest and breed on the provided carcasses. Two size measurements were taken, one of the elytra and the other of the pronotum. During each check, burial status of the carcasses was noted being either one of following: Surface, $\frac{1}{4}$ Buried, $\frac{1}{2}$ Buried, $\frac{3}{4}$ Buried, or Buried. This process was repeated as cans were checked daily for nesters and those beetles with markers already on them, re-nesters, were recorded. The process was repeated for approximately four weeks, allowing for one re-baiting of each study site.

3. Results

262 individuals were collected from both species (*N. investigator* and *N. defodiens*). The majority of individuals collected were *N. investigator*. Of these, 249 were *N. investigator* and 13 were *N. defodiens*. All *N. defodiens* were excluded from data analysis since none were successful in winning a carcass during either rounds of baiting. Thus, a variance in the number of broods produced by species was unable to be evaluated. Elytra and pronotum size distribution of all *N. investigators* measured exhibited a normal distribution (Elytra: Mean = 9.33 mm, N = 249; Pronotum: Mean = 5.67 mm, N = 249) (Figure 1 and 2). Breeders' sizes were close to the mean for both elytra and pronotum (Mean Size of Breeder Elytra = 9.48 mm, Mean Size of Breeder Pronotum = 5.73 mm).

Of the 30 cans baited during the first round of collection at the Research Meadow site, only two successfully produced broods. Of the cans that did not contain broods, most rodent carcasses were found mummified on the surface of the soil. This is likely the result

of the consistent heat and lack of precipitation that persisted for four days during July when the cans were not checked or watered. *Thanatophilus lapponicus* (northern carrion beetle) had also claimed several of the carcasses that remained on the surface of the soil for several days. Of the 20 cans baited during the first round of collection at the Barr Meadow site, again, only two successfully produced broods. The remaining cans also contained either eaten or mummified rodent carcasses or had been claimed by *T. lapponicus*. Often, carcasses were buried and abandoned when overrun with maggots and other fly larvae.

During the first round of collection at the Research Meadow site, all adults to arrive at a carcass were marked with two triangular cuts on their left elytra. This method was quickly abandoned as it was time consuming and it was only necessary to mark those adults who successfully nested with the carcass. At the Research Meadow site, several adults who were marked with the two triangular cuts during the first round of data collection reappeared during the second round on new carcasses. Although none were successful in breeding during either of the rounds, it is interesting to note the reoccurrence of these beetles at the site. Furthermore, in a different study being conducted by Dr. Rosemary Smith at the Kettle Ponds south of Gothic, two beetles were found in census traps with two triangular markings on their left elytra. This is indicative of the distance burying beetles are capable of traveling which has been said to be remarkable for a beetle species (Eggert and Muller 1997, Merrick & Smith 2004).

Furthermore, three cans were raided during the second round of baiting at the Research Meadow site by a fox that lives within the Gothic townsite. One of the wire grids on a different can had fecal remains sitting on top of it; most likely believed to be from the fox as well. This also occurred twice during the second round of baiting at the Barr Meadow site, though no cans were raided there. No beetles successfully produced broods at the Barr Meadow site during the second round of baiting. Interestingly however, one female to arrive at a carcass at this site was marked as a breeder from the first round, though she did not successfully reproduce a second time. 4 broods were produced during the second round of baiting at the Research Meadow site. None of the breeders at this site were those marked during the first round.

4. Discussion

Evaluating the number of carcasses offered at each site during each round in comparison with the number of carcasses located, buried as well as those that yielded broods produced the most interesting results (Figure 3). The number of carcasses offered was significantly larger than the number of carcasses actually bred on. Of the 29 (Research Meadow) or 20 carcasses (Barr Meadow) offered during the first round of baiting, only 4 broods were produced at each of the sites. However, 97 beetles visited carcasses at the Research Meadow site and 72 visited the Barr Meadow site, revealing competition to be a strong biological limitation to be considered during the carcass acquisition process.

The number of carcasses buried was three times greater than the number of carcasses to actually yield broods at the Research Meadow site during the first round of baiting. At the Barr Meadow site, this number was twice as large as the number of carcasses that yielded broods. These proportions increased further during the second round of baiting which occurred later in the breeding season in early August, with almost twice as many carcasses buried than carcasses that actually yielded broods at the Research Meadow site

and six times as many carcasses buried than carcasses that actually yielded broods at the Barr Meadow site. Several factors can explain this variation. The first being environmental conditions during the breeding process. Temperatures during both the first and second rounds of baiting increased for several days after carcasses were buried. No precipitation occurred immediately after the carcasses were buried, adding to the already dry and hot conditions. Although periodic watering of the cans was attempted, cans were often left on their own during the weekends without checks or watering. Once cans were checked the following weeks, rodent carcasses were often found mummified or overrun with *T. lapponicus*. Also, buried carcasses were often abandoned before a brood ball was completed. This is also most likely the result of environmental conditions since soil was often extremely dry in the cans found without beetles or broods.

Competition with *T. lapponicus* was considered in light of its presence on several of the carcasses during each round of baiting at each of the sites. Although it is possible that *T. lapponicus* won the carcasses from *N. investigator*, it is highly unlikely as shown by previous competition studies (Cloyed et al. 2008) as well as most likely being the result of environmental factors at play. Cans found with mummified carcasses were just as likely to have *T. lapponicus* as those with carcasses that were never discovered by *N. investigator*. This is an indication that it is the presence of *N. investigator* influencing the presence of *T. lapponicus*. Furthermore, it has been previously shown that the number of *T. lapponicus* present at a given carcass has no effect on the success of *Nicrophorus* spp. in winning a given carcass (Cloyed et al. 2008). Thus, *T. lapponicus* was not considered as an important factor when data was analyzed.

In conclusion, although these results might suggest otherwise, it is quite possible that *N. investigator* is iteroparous. A serious flaw in the experimental design was not detected until after the experiment was completed. It was not logistically possible to test for multiple broods per season with only two rounds of baiting. The number of beetles actually producing broods during either round was so much lower than the number of beetles actually visiting carcasses that the chances of detection a second time was incredibly low, shown by the fact that only one breeder was detected a second time.

In the future, given that *N. investigator*'s breeding season lasts for approximately eight weeks and requires approximately two weeks to successfully produce a brood, four rounds of baiting with carcasses should be placed out in a stronger attempt to detect repeat breeders. Also, serious consideration should be given to the breeding habitat to ensure protection from potential carnivores able to locate rodent carcasses as well as to ensure protection from the sun, since it takes only a few days with no precipitation and high temperatures for the soil within the cans to dry out. The results of this study as well as these factors taken into consideration should allow for a greater success in future studies of iteroparity among burying beetles.

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7. Figures

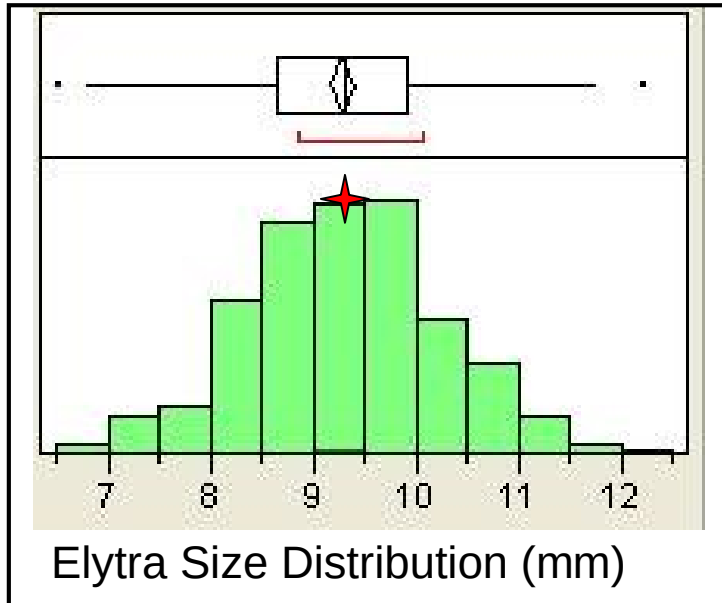


Figure 1. Elytra Size Distribution (mm).

A distribution of elytra size (mm) (Mean = 9.33 mm, N = 249). Red star indicates mean elytra size of breeders (Mean = 9.48 mm).

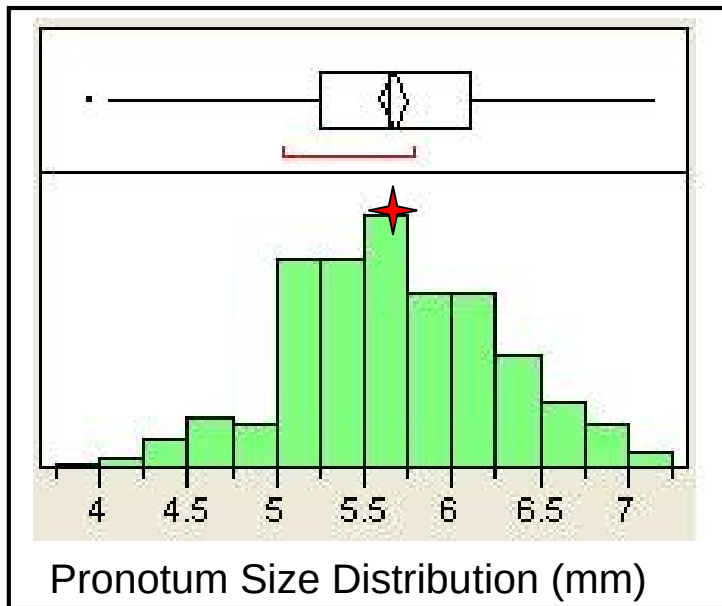


Figure 2. Pronotum Size Distribution (mm).

A distribution of pronotum size (mm) (Mean = 5.67 mm, N = 249). Red star indicates mean pronotum size of breeders (Mean = 5.73 mm).

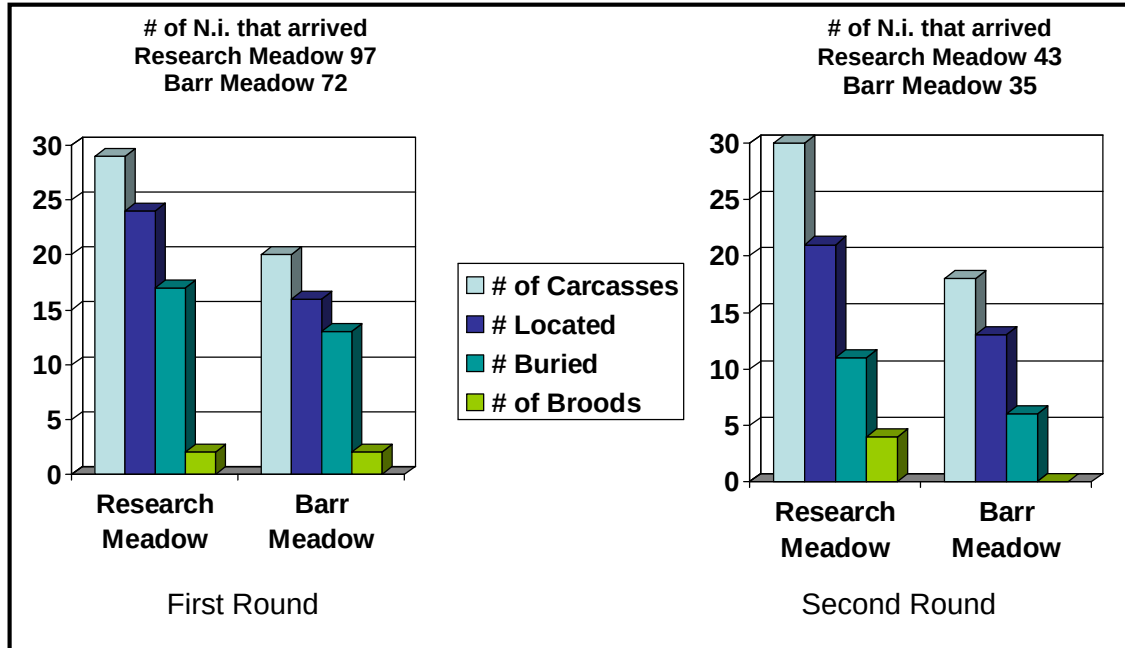


Figure 3. First and Second Round Results.

After the number of *N. investigator*'s to arrive at each site was separated, data was analyzed further. The number of carcasses located, buried as well as the number of broods produced at each site was compared to the total number of carcasses offered at each site during each round of baiting.