

Evidence for differential diel activity patterns in two co-occurring species of burying beetles (Coleoptera: Silphidae: Nicrophorinae)

LINDSEY M. COOK^{1,*}, ASHLEE N. SMITH¹, PETER J. MEYERS^{1,3}, J. CURTIS CREIGHTON², AND MARK C. BELK¹

¹Department of Biology, Brigham Young University, Provo, UT

²Department of Biological Sciences, Purdue University, Northwest, Hammond, IN

³Present address: Department of Biological Sciences, University of Notre Dame, Notre Dame, IN

ABSTRACT.—Multiple mechanisms have been suggested to facilitate coexistence among species of burying beetles, including differential use of habitat and food resources. *Nicrophorus marginatus* and *Nicrophorus guttula* are similarly sized burying beetles that co-occur in many parts of their range. We used observations to quantify and compare their diel activity patterns and tested for possible niche partitioning between the species. We monitored activity of individuals of each species over four 24-h periods. Both species exhibit bimodal activity patterns with peaks of activity in the morning and evening. *Nicrophorus guttula* is active earlier in the morning and later in the evening than *N. marginatus*. This difference suggests possible temporal niche partitioning and may represent an important mechanism for the coexistence of these burying beetle species.

RESUMEN.—Se han sugerido múltiples mecanismos, para facilitar la coexistencia entre las especies de escarabajos enterradores, entre los que incluyen el uso diferencial del hábitat y de los recursos alimenticios. *Nicrophorus marginatus* y *Nicrophorus guttula* son escarabajos enterradores de tamaño similar que coexisten dentro de su rango de distribución. Por medio de observaciones cuantificamos y comparamos los patrones de actividad, y analizamos posibles divisiones de nichos entre las especies. Monitoreamos la actividad de los individuos de cada especie durante cuatro períodos de 24 horas. Ambas especies mostraron patrones de actividad bimodales con picos de actividad en la mañana y en la tarde. El escarabajo *Nicrophorus guttula* es más activo a principios de la mañana y al final de la tarde que *N. marginatus*. Esta diferencia indica una posible división temporal de nichos, pudiendo representar un mecanismo importante para la coexistencia de estas especies de escarabajos enterradores.

Burying beetles, genus *Nicrophorus*, are broadly distributed in the northern hemisphere (Sikes and Venables 2013, Sikes et al. 2016). In many locations, multiple species co-occur (Peck and Kaulbars 1987, Lomolino et al. 1995, Dekeirsschieter et al. 2011) and compete for small vertebrate carcasses that all *Nicrophorus* species use for reproduction (Scott 1998). Typically, co-occurring species that have similar resource requirements exhibit differences in some aspects of their niche to reduce competitive overlap (i.e., niche partitioning). Niche partitioning has been documented among some species of co-occurring burying beetles (Wilson et al. 1984, Trumbo 1990, Beninger and Peck 1992, Scott 1998, Ikeda et al. 2006); however, the ecology and behavior of most species of burying beetles is poorly described.

Nicrophorus guttula and *N. marginatus* are closely related species of burying beetles (Sikes and Venables 2013) that occupy grassland habitats in western North America (Peck and Anderson 1985, Peck and Kaulbars 1987). Both species are considered to be diurnal (Peck and Kaulbars 1987, Bedick et al. 2006), and they successfully reproduce under laboratory conditions on carcasses that range from 5 to 50 g (Meyers 2014), suggesting the potential for competition between species for similarly sized carcasses. This similarity in both habitat use and resource use (i.e., small vertebrate carcasses) suggests that *N. guttula* and *N. marginatus* may exhibit some difference in activity patterns that might somewhat alleviate high levels of competition. We tested this prediction by quantifying and comparing diel activity patterns of both species in a controlled environment.

*Corresponding author: byulindsey@gmail.com

LMC  orcid.org/0000-0003-4827-4601

ANS  orcid.org/0000-0001-8941-8574

PJM  orcid.org/0000-0002-4305-7934

JCC  orcid.org/0000-0001-8032-7318

MCB  orcid.org/0000-0002-0576-0717

METHODS

To generate the laboratory populations for this experiment, we captured adult *N. marginatus* and *N. guttula* at the Goshen Warm Springs Recreation Area near Goshen, Utah, in the summer of 2012 using pitfall traps baited with aged chicken meat. We placed wild-caught pairs on 30-g mouse carcasses and allowed them to breed. We recorded the date of eclosion for all first-generation laboratory-bred beetles and designated this as the first day of life. We placed newly eclosed individuals in small clear plastic containers ($15.6 \times 11.6 \times 6.7$ cm), fed them raw chicken liver *ad libitum* twice weekly, and kept them in an environmental room. The 10×14 -ft. (3×4.3 -m) environmental room was temperature controlled at 21 °C with a positive pressure system to minimize changes in air currents. The room was maintained on a schedule of 12 h of full daylight (from 08:00 to 20:00), 1 h each of reduced light in the morning (07:00 to 08:00) and evening (20:00 to 21:00), and 10 h of darkness. The lights in the environmental room were full-spectrum fluorescent lights. These conditions simulated the natural light/dark pattern consistent with the beetles' summer breeding season in their natural environment.

To determine the diel activity patterns, we randomly chose 20 male and 20 female beetles ($n = 40$ for both species) of each species to use in this experiment. We used equal numbers of males and females from each species, but we did not assess differences between sexes in this experiment. We considered all individuals as independent replicates for analysis. All beetles were between 25 and 45 d old, an age at which all individuals are sexually mature but prior to the onset of senescence. To begin the observation period, we placed each beetle in a lidded transparent container with 1 cm of topsoil and a moist paper towel. The soil and paper towel were used to prevent desiccation and to simulate shallow soil or leaf litter for the beetles to rest under during periods of inactivity. We started observations after a 4-h acclimation period. We observed beetle activity through the transparent containers (lids were not opened during observations) once per hour for 4 consecutive days (24 h each day for a total of 96 h), and we recorded whether each beetle was active or inactive at

that time. We recorded a beetle as active if it was on top of the soil and moving around but inactive if it was buried under the soil or in the paper towel. It is possible that beetles could have been active, in some way, under the soil and paper towels. However, competitive interactions among burying beetles in the absence of a carcass are all aboveground activities. Any activity occurring below ground, defined in circadian rhythm or otherwise, does not result in any sort of competitive interaction and is therefore outside the scope of this experiment. Thus, we defined activity as aboveground activity, and based our conclusions on that definition. All observations were made without touching or opening the containers in order to ensure that beetles were not unduly disturbed by any air currents, temperature changes, or vibrations as the result of our presence in the environmental chamber. We maintained the same light/dark cycle as described above for the duration of the experiment, using red light for nocturnal observations to avoid disrupting the normal activity pattern. To avoid inducing activity, we did not feed beetles during the observation period. Both species received the same procedure with the same precautions to minimize disturbance.

We designed the experiment as a repeated measure of activity over a 24-h day. Because we scored individual activity as either active or inactive, the response variable was the proportion of the 40 beetles of each species that were active at each hour. We included species (2 levels), time (24 levels), and the interaction between species and time as predictor variables. Each beetle was observed for 4 consecutive days (24-h periods), so we included the number of the day (1 through 4) as a random effect in the model. We evaluated the response variables for normality of residuals and equal variances. The data met these assumptions. We used the procedure MIXED in SAS (SAS 9.3, SAS Institute, Cary, NC, USA) for the analysis.

RESULTS

Activity rate data for both species (collective) differed significantly across the 24-h period (time main effect, Table 1); and activity rate differed significantly between species across the 24-h period (time \times species interaction effect, Table 1). Both species exhibited

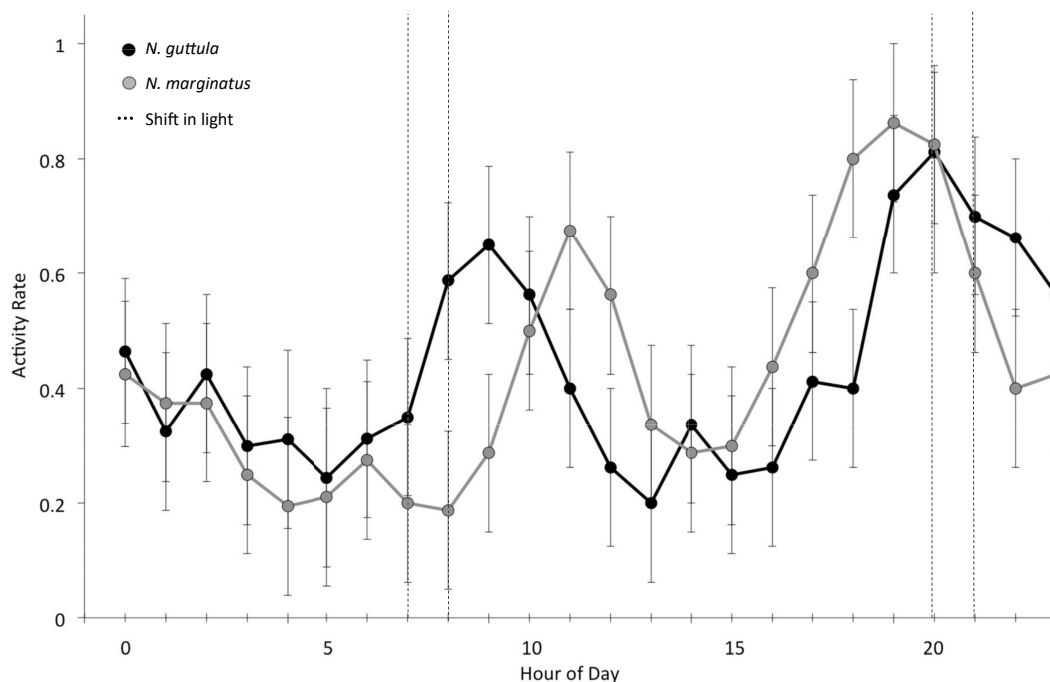


Fig. 1. Mean ($\pm 95\%$ CI) proportion of individuals of *Nicrophorus guttula* and *N. marginatus* that were active over a 24-h period (averaged over four 24-h periods). Shifts from periods of darkness, full light (08:00–20:00) and reduced light (07:00–8:00 and 20:00–21:00) are indicated by dashed lines.

TABLE 1. Mixed-model analysis of variance (ANOVA) table for the effect of species, time of day, and the interaction between species and time of day on activity levels in *Nicrophorus guttula* and *N. marginatus*. Significant *P* values are bolded.

Effect	num df/den df	<i>F</i>	<i>P</i>
Species	1/139	0.10	0.7517
Time of day	23/139	13.22	<0.0001
Species \times time of day	23/139	4.74	<0.0001

bimodal activity patterns with peaks in the morning and late afternoon. However, *N. guttula* was active earlier in the morning and later in the evening than *N. marginatus* (Fig. 1).

In their daily activity patterns, *N. guttula* and *N. marginatus* both demonstrate clear bimodal patterns of activity. They are most active in the morning and late afternoon, with relatively little activity during midday and at night. Within these bimodal patterns, *N. guttula* is active earlier in the morning and later in the evening than *N. marginatus*. Previous studies have characterized both *N. marginatus* and *N. guttula* as diurnally active (Peck and Kaulbars 1987, Bedick et al. 2006). Our data suggest that in our location, both species are active during periods of reduced light in the

evening as well. It is important to note that activity patterns among burying beetles may vary by geographical location and time of year. It is likely that burying beetles collected at other locations and observed at different times of the year have different activity patterns. More research can be done to describe general activity patterns among populations of *N. marginatus* and *N. guttula* across a broader spectrum of conditions. Our data provide a set of observations to which comparisons of activity under different conditions and in different locations can be made (using similar, easy-to-implement methodologies).

Another pair of co-occurring burying beetle species, *N. orbicollis* and *N. defodiens*, have

similar bimodal patterns of activity (Trumbo and Bloch 2002). *Nicrophorus orbicollis* and *N. defodiens* occupy similar habitats and are often found competing for the same carcasses. Similar to the pattern we describe here, the smaller beetle, *N. defodiens*, becomes active several hours before the larger *N. orbicollis* (Trumbo and Bloch 2002). This suggests that size may play a significant role in the different daily activity patterns exhibited by competing burying beetles. It is likely that larger species (such as *N. marginatus* and *N. orbicollis*) are able, through competitive interactions, to relegate smaller species (*N. guttula* and *N. defodiens*) to be active at different times of the day. The differences in activity patterns of co-occurring *Nicrophorus* beetles may represent a form of temporal niche partitioning, which may reduce competitive interactions between species. While our experiment does not conclusively prove niche partitioning, our results suggest that temporal niche partitioning may facilitate coexistence between these 2 co-occurring species. It would be interesting to compare activity patterns of these 2 species from allopatric locations. If larger species do cause a shift in the diel activity pattern of smaller species in sympatry, then activity patterns of smaller species may be different in allopatric populations.

Alternatively, activity patterns may be related to temperature tolerances and sensitivity to desiccation rather than interspecific competition. *Nicrophorus marginatus* loses water quickly at high temperatures and thus might remain inactive during the hottest part of the day to avoid desiccation (Bedick et al. 2006). Additionally, *N. marginatus* and *N. guttula* may differ in their thermoregulatory abilities, which would allow them to occupy different temperature niches. Organisms, including beetles, tend to concentrate their activity to times that provide a thermal optimum (Hamilton 1971, Dreisig 1980). Thermal optimums vary depending on food availability and species' thermoregulatory capacity (Dreisig 1980). Within the genus *Nicrophorus*, different species exhibit varying environmental temperature tolerances (Wilson et al. 1984, Trumbo 1990, Sikes 1996, Scott 1998). Body sizes and morphological features of burying beetles play a significant role in their ability to thermoregulate (Merrick and Smith 2004). In general, larger beetle species with lower

surface-area-to-volume ratios have a higher capacity for thermoregulation (Merrick and Smith 2004) at higher temperatures. Additionally, *N. orbicollis* and *N. defodiens* have differential success in securing carcasses depending on temperature, which provides support for this hypothesis. On warm nights, *N. orbicollis* has greater success securing carcasses, but on cool nights, *N. defodiens* shows greater success (Wilson et al. 1984). According to this argument, *N. marginatus* may be active later in the morning and earlier in the evening than *N. guttula* because of its greater capacity for thermoregulation at high temperatures. However, both species probably avoid being active during the hottest part of the day to avoid desiccation. Further research could be done to compare temperature tolerances of these species and to test their activity patterns in response to variable temperatures.

ACKNOWLEDGMENTS

This research was supported by the Department of Biology at Brigham Young University. We thank J. Rehm and M. Segura for help with the experimental observations.

LITERATURE CITED

- BEDICK, J.C., W.W. HOBACK, AND M.C. ALBRECHT. 2006. High water-loss rates and rapid dehydration in the burying beetle, *Nicrophorus marginatus*. *Physiological Entomology* 31:23–29.
- BENINGER, C.W., AND S.B. PECK. 1992. Temporal and spatial patterns of resource use among *Nicrophorus* carrion beetles (Coleoptera: Silphidae) in a sphagnum bog and adjacent forest near Ottawa, Canada. *Canadian Entomologist* 124:79–86.
- DEKEIRSSCHIETER, J., F. VERHEGGEN, G. LOGNAY, AND E. HAUBRIGE. 2011. Large carrion beetles (Coleoptera, Silphidae) in western Europe: a review. *Biotechnology Agronomy Society and Environment* 15:435–447.
- DREISIG, H. 1980. Daily activity, thermoregulation and water-loss in the tiger beetle *Cicindela hybrida*. *Oecologia* 44:376–389.
- HAMILTON, W.J. 1971. Competition and thermoregulatory behavior of Namib Desert tenebrionid beetle genus *Cardiosis*. *Ecology* 52:810–822.
- IKEDA, H., K. KUBOTA, T. KAGAYA, AND T. ABE. 2006. Niche differentiation of burying beetles (Coleoptera: Silphidae: Nicrophorinae) in carcass use in relation to body size: estimation from stable isotope analysis. *Applied Entomology and Zoology* 41:561–564.
- LOMOLINO, M.V., J.C. CREIGHTON, G.D. SCHNELL, AND D.L. CERTAIN. 1995. Ecology and conservation of the endangered American burying beetle (*Nicrophorus americanus*). *Conservation Biology* 9:605–614.
- MERRICK, M.J., AND R.J. SMITH. 2004. Temperature regulation in burying beetles (*Nicrophorus* spp.:

- Coleoptera: Silphidae): effects of body size, morphology and environmental temperature. *Journal of Experimental Biology* 207:723–733.
- MEYERS, P.J. 2014. Variation in resource utilization and cost of reproduction for two burying beetle species. Master's thesis, Brigham Young University, Provo, UT.
- PECK, S.B., AND R.S. ANDERSON. 1985. Taxonomy, phylogeny, and biogeography of the carrion beetles of Latin America (Coleoptera: Silphidae). *Quaestiones Entomologicae* 21:247–314.
- PECK, S.B., AND M.M. KAULBARS. 1987. A synopsis of the distribution and bionomics of the carrion beetles (Coleoptera: Silphidae) of the conterminous United States. *Proceedings of the Entomological Society of Ontario* 118:47–81.
- SCOTT, M.P. 1998. The ecology and behavior of burying beetles. *Annual Review of Entomology* 43:595–618.
- SIKES, D.S. 1996. The natural history of *Nicrophorus nigrita*, a western Nearctic species (Coleoptera: Silphidae). *Pan Pacific Entomologist* 72:70–81.
- SIKES, D.S., S.T. TRUMBO, AND S.B. PECK. 2016. Cryptic diversity in the New World burying beetle fauna: *Nicrophorus hebes* Kirby; new status as a resurrected name (Coleoptera: Silphidae: Nicrophorinae). *Arthropod Systematics Phylogeny* 74:299–309.
- SIKES, D.S., AND C. VENABLES. 2013. Molecular phylogeny of the burying beetles (Coleoptera: Silphidae: Nicrophorinae). *Molecular Phylogenetics and Evolution* 69:552–565.
- TRUMBO, S.T. 1990. Reproductive success, phenology and biogeography of burying beetles (Silphidae, *Nicrophorus*). *American Midland Naturalist* 124:1–11.
- TRUMBO, S.T., AND P.L. BLOCH. 2002. Competition between *Nicrophorus orbicollis* and *N. defodiens*: resource locating efficiency and temporal partitioning. *Northeastern Naturalist* 9:13–26.
- WILSON, D.S., W.G. KNOLLENBERG, AND J. FUDGE. 1984. Species packing and temperature dependent competition among burying beetles (Silphidae, *Nicrophorus*). *Ecological Entomology* 9:205–216.

Received 4 December 2018

Accepted 18 December 2018

Published online 24 June 2019