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# A novel association between a beetle and a snake: Parasitism of *Elaphe obsoleta* by *Nicrophorus pustulatus*[1]

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*Abstract*: While studying the nesting ecology of black rat snakes (*Elaphe obsoleta* [Say]; Serpentes: Colubridae) in eastern Ontario, we discovered that their eggs regularly contained larvae of the burying beetle *Nicrophorus pustulatus* (Herschel) (Coleoptera: Silphidae). The beetle appears to be a parasitoid of the snakes, which may make this the first documented case in which a vertebrate is the host of a parasitoid. Up to 100% of snake eggs in a nest were destroyed by beetles, indicating that *N. pustulatus* may be a significant, and heretofore unrecognized source of egg mortality for oviparous snakes. Evidence suggests that the association between these two species is well established, and that the beetle may also attack other species of oviparous snakes. Identification of snake eggs as the substrate for reproduction by *N. pustulatus* solves the mystery of where this species breeds. Also, the large amount of available biomass in communal black rat snake nests may explain why *N. pustulatus* is so fecund relative to its congeners.
*Keywords*: black rat snake, *Elaphe obsoleta*, burying beetle, *Nicrophorus pustulatus*, parasitoid, eggs.

*Résumé* : Au cours d'une étude écologique de la nidification de la couleuvre obscure (*Elaphe obsoleta* [Say]; Serpentes: Colubridae) dans l'est de l'Ontario, nous avons découvert que leurs œufs contenaient régulièrement des larves du scarabée enfouisseur *Nicrophorus pustulatus* (Herschel) (Coleoptera: Silphidae). Le scarabée est un parasitoïde des serpents et nos observations semblent mettre en lumière le premier cas où un vertébré est l'hôte d'un parasitoïde. Jusqu'à 100 % des œufs de serpents contenus dans un nid peuvent être détruits par les scarabées, démontrant que *N. pustulatus* peut représenter une source de mortalité importante, précédemment insoupçonnée, des œufs de serpents ovipares. Plusieurs observations suggèrent que l'association entre ces deux espèces est bien établie et que les scarabées attaquent sans doute d'autres espèces de serpents ovipares. L'identification des œufs de serpents comme substrat de reproduction pour *N. pustulatus* résout le mystère entourant le lieu de reproduction de cette espèce. De plus, l'importante quantité de biomasse disponible dans les nids communautaires des couleuvres obscures explique pourquoi *N. pustulatus* est si fécond comparativement à ses congénères.
*Mots-clés* : couleuvre obscure, *Elaphe obsoleta*, scarabée enfouisseur, *Nicrophorus pustulatus*, parasitoïde, œufs.

## Introduction

Identifying and quantifying the impact of major mortality factors is fundamental to understanding the biology of animal populations. The mortality factors affecting snake populations are generally poorly known, particularly those affecting snakes early in life (Parker & Plummer, 1987). The black rat snake (*Elaphe obsoleta* [Say]) is no exception, and because this species is in need of conservation (Prior, Gibbs & Weatherhead, 1997), identifying its principal sources of mortality is particularly important. Here we report evidence suggesting that the burying beetle *Nicrophorus pustulatus* (Herschel) may be an important cause of egg mortality for black rat snakes.

*Nicrophorus* species typically reproduce by laying eggs in the soil near small vertebrate carcasses they have buried and prepared (Scott, 1998). *Nicrophorus* species have been well studied because of their interesting communal and parental care behaviours. However, *N. pustulatus* remains enigmatic. Typical of the genus, *N. pustulatus* buries small mammal carcasses in the laboratory and reproduces successfully. However, the species has never been found to bury similar carcasses placed experimentally in the wild (Robertson, 1992; Scott, 1998). Also, *N. pustulatus* is approximately three times more fecund than congeners on large (rat-sized) carcasses in captivity (Trumbo, 1992). This high fecundity has led to the hypothesis that *N. pustulatus* must breed on large carcasses in the wild, although no evidence in support of this hypothesis has been obtained (Trumbo, 1992; Scott, 1998). The observations we report here solve both the mystery of where *N. pustulatus* breeds and why it is so fecund.

## Material and methods

Observations on the association of *N. pustulatus* with snake eggs were made as part of a long-term study of the population biology of black rat snakes at the Queen's University Biological Station, approximately 40 km north of Kingston, Ontario. From 1997 to 1999 we implanted radio transmitters in a total of 35 adult female black rat snakes captured between emergence from hibernation in late April to early May (Blouin-Demers, Prior & Weatherhead, 2000) and egg laying in July. To implant transmitters we modified the surgical technique described by Reinert & Cundall (1982). This involved using sterile techniques to surgically implant a radio-transmitter in the body cavity of the snake, suturing the transmitter to a rib to prevent its

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migration inside the coelom, and leaving the antennae between the epidermis and the outer body wall. For the surgery we anesthetized snakes using isoflurane delivered via a precision vaporiser. Fourteen of the 35 females reproduced in the year they were tracked, allowing us to follow them to their nesting sites. Because female black rat snakes often lay their eggs in communal nests, many of the females tracked led us to the same nests.

Of the eight nests we discovered, seven were accessible. We excavated these seven nests in 1998 and 1999 and removed both freshly laid eggs and egg shells remaining from previous years. We initially excavated a nest when the female we had tracked to the nest emerged and a sharp decline in her body weight indicated she had laid eggs. We subsequently excavated nests on a regular basis to obtain eggs laid by other females. Following each excavation we returned nests as close to their original condition as possible. Subsequent egg laying in these nests indicated that the disturbance did not deter other snakes from using these nests.

Our original purpose in excavating nests was to obtain information on black rat snake clutch sizes and to incubate eggs to collect data on juvenile rat snakes. However, upon discovering beetle larvae in rat snake eggs and adult beetles in the nests, we systematically documented the occurrence of the beetles. We examined a total of 300 fresh snake eggs and 153 egg shells from previous years. Beetle damage to fresh eggs was evident from holes (12.5 ± 4.6 mm in diameter) and larva in eggs, or smaller holes (2.6 ± 0.8 mm in diameter) possibly made by adult beetles to feed and/or to allow larvae access to eggs. We dissected 11 eggs containing beetle larvae. We considered old eggs to have been destroyed by beetles when shells had small circular holes instead of large tears produced by the egg tooth of hatching snakes. We collected several specimens of both larval and adult beetles for identification. All animals were handled and cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care and our experimental procedures had been approved by the Carleton University Animal Care Committee.

## Results and Discussion

We found adult beetles in three of the seven nests we excavated, and beetle larvae and/or evidence of beetle damage to eggs in six of seven nests (Table I). Dissection of eggs containing beetle larvae confirmed that the snake embryos were still alive in some of the eggs, so otherwise viable eggs were being attacked. Overall damage levels were high (up to 100% of eggs destroyed), and average levels could have been higher had we not removed intact eggs when we excavated nests. All adult and larval beetle specimens were identified as *N. pustulatus* by Dr. Robert Anderson of the Canadian Museum of Nature.

The association between the beetles and the snakes appears to satisfy the criteria of being a parasitoid-host relationship. Parasitoids differ from other parasites because they are only parasitic as a larva, they are relatively large in comparison with their host, and they always kill their host; they differ from predators because each parasitoid larva or group of larvae only attack and kill a single host individual rather than several (Noble & Noble, 1971). Because known parasitoids are insects (primarily hymenopterans and dipterans) and their hosts are arthropods, the definition of parasitoid is often restricted to those taxa (Godfray & Hassell, 1991; May, 1991). Thus, a burying beetle that is a parasitoid of black rat snakes is a novel association, and to our knowledge represents the first documented evidence of a vertebrate being the host of a parasitoid.

TABLE I. Incidence of parasitism by *Nicrophorus pustulatus* in black rat snake eggs. Missing values for 1998 are for nests found in 1999

| Nest | Percent parasitism (Number of eggs per nest) | | | Number of adult beetles per nest | |
|---|---|---|---|---|---|
| | Old eggs | New eggs 1998 | New eggs 1999 | 1998 | 1999 |
| 1 | 100.0 (12) | 22.7 (22) | 16.0 (25) | 3 | 4 |
| 2 | 66.7 (12) | 12.0 (25) | (0) | 2 | 0 |
| 3 | 30.4 (46) | 30.8 (52) | (0) | 2 | 0 |
| 4 | (0) | 0.0 (15) | (0) | 0 | 0 |
| 5 | 27.3 (11) | 0.0 (15) | 0.0 (12) | 0 | 0 |
| 6 | 16.7 (72) | | 0.0 (111) | | 0 |
| 7 | (0) | | 0.0 (23) | | 0 |

Note: Nests 1-3 were located in hollow, standing dead trees, nest 4 in a fallen dead tree, and nests 5-7 in piles of decaying vegetation. Nests with more than 15 eggs represent more than one clutch. In each beetle assemblage found, there was always a single male and one or more females.

Identifying rat snake eggs as a food source for *N. pustulatus* not only solves the mystery of what *N. pustulatus* uses as a breeding resource, but may also explain why the species is so fecund. Rat snakes often nest communally, and with an average clutch mass of 152 g ($N$ = 41: G. Blouin-Demers, unpublished data), the egg biomass in a nest with the clutches of only two female rat snakes easily exceeds the 230 g carcasses that produced large *N. pustulatus* broods in captivity (Trumbo, 1992). It may be noteworthy that, at least for our observations of freshly laid eggs, only nests with more than one clutch of rat snake eggs were attacked by the beetles. If further observations substantiate this pattern, then the question arises as to whether communal nests are more easily located by the beetles, or whether they prefer these nests because they contain more resources.

*N. pustulatus* may have become parasitic by first exploiting snake eggs that failed to hatch. Digging 15-45 cm in loose organic material to access snake eggs is consistent with digging exhibited by members of the genus when digging to locate and access carcasses that have been buried and prepared by other beetles (Trumbo, 1994). Our observations, the failure of *N. pustulatus* to come to small carcasses placed experimentally in the field, and *N. pustulatus*' high fecundity in the laboratory, all indicate that the evolution of *N. pustulatus* as a parasitoid of oviparous snakes is not a recent event. In addition, reports of beetle larvae in black rat snake eggs in Illinois (L. Keller, pers. comm.) and in fox snake (*Elaphe vulpina*) eggs in southern Ontario (R. Willson, pers. comm.) are sufficiently similar to our observations to suspect they also involve *Nicrophorus* species, and most likely *N. pustulatus*. Thus, *N. pustulatus* may not only be a specialist parasitoid of rat snake eggs, but may attack the eggs of many oviparous snake species. However, some of the northern distribution records of *N. pustulatus* fall outside the range of oviparous snakes (Anderson & Peck, 1985). Thus, *N. pustulatus* may still exploit vertebrate carcasses in parts of its range, explaining the performance of ancestral behaviours in captivity. Alternatively, turtles might serve as hosts where snakes are unavailable.

*N. pustulatus* is widespread in eastern North America but is considered rare relative to other *Nicrophorus* species (Anderson & Peck, 1985). Our observations suggest that this species is not rare in nests of black rat snakes. Thus, this species may be recorded more frequently as researchers begin to look for it in association with snake nests. Should this be the case, then *N. pustulatus* may prove to be an important factor in the population biology of some snakes. Establishing the importance of *N. pustulatus* is particularly relevant for species such as *Elaphe obsoleta*, which are of conservation concern (Prior, Gibbs & Weatherhead, 1997).

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