Host shift by the burying beetle, *Nicrophorus pustulatus*, a parasitoid of snake eggs

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Abstract

Recent work [Ecoscience (2000) vol. 7, 395–397] suggests that the burying beetle *Nicrophorus pustulatus* may have undergone a remarkable host shift, exploiting snake eggs rather than carrion as resources for breeding. We conducted behavioural and physiological experiments to examine the hypothesis of a host shift and to formulate hypotheses on its origin. Two congeners of *N. pustulatus*, *Nicrophorus orbicollis* and *Nicrophorus defodiens* did not respond to snake eggs with typical breeding behaviour. When *N. pustulatus* male–female pairs ($n = 14$) were presented with clutches of snake eggs, the number of offspring but not the mean size of offspring varied with snake egg mass, indicating effective regulation of brood size. When breeding on turtle eggs, *N. pustulatus* had a more variable response than when exploiting snake eggs, suggesting that turtle eggs are not a primary resource for breeding. *Nicrophorus pustulatus* presented with both snake eggs and a mouse carcass combined and exploited the two resources within the same nest (10 of 12 trials). Mouse carcasses and snake eggs were treated differently. Carcasses were moved, buried and stripped of hair in a manner characteristic of burying beetles, whereas snake eggs were not moved or buried. Females that discovered a mouse carcass also had a significantly greater juvenile hormone increase than did females discovering snake eggs. Some responses to the two resources, however, were similar. Female *N. pustulatus* oviposited rapidly in response to either a mouse carcass or snake eggs, and males elevated sex pheromone emission in response to either resource. The efficient use of snake eggs, the ability to regulate brood size and the different responses to snake eggs and carrion suggest that *N. pustulatus* is well adapted to exploiting snake eggs for breeding. The use of snake eggs by *N. pustulatus* has potential implications for conservation of oviparous reptiles.

Introduction

A host shift is a key evolutionary process that has been recognized to be important in niche differentiation and sympatric speciation in diverse groups including symbio-

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nts (Antonovics *et al.*, 2002; Lopez-Villavicencio *et al.*, 2005), phytophagous insects (Feder *et al.*, 1995; Rossbach *et al.*, 2005) and insect parasitoids (Brown *et al.*, 1995; Hayward & Stone, 2006). Host shifts are typically between host species that are ecologically or phylogenetically related (Messina, 2004a; Rossbach *et al.*, 2005), or in the case of myiasis-inducing dipterans, between dead and living tissue of the same host species (Stevens *et al.*, 2006). For a host shift to evolve, organisms must respond to a new set of host-related cues

(Olsson *et al.*, 2006; Faucci *et al.*, 2007; McBride, 2007) and in some cases, actively avoid cues associated with the ancestral host (Forbes *et al.*, 2005). As host choice becomes more selective during a host shift, selection for specializations to exploit the new host can evolve (Kawecki, 1998; Messina, 2004b; Egan & Funk, 2006); sub-optimal performance on a host may indicate a recent host shift (Lopez-Villavicencio *et al.*, 2005). Host selection, however, is not always a single-step process. Many insect parasitoids, for example, use general cues to locate the approximate location of a host, and then use more host-specific cues at close range (Combes, 2005). It may be possible to achieve a high degree of host specialization by altering one aspect of host choice behaviour (e.g. long-range searching) while leaving other aspects of host choice behaviour intact (e.g. response to host after location). It is also possible that when parents make a definitive host choice, selection on offspring to distinguish between hosts may be weak.

Recently, Blouin-Demers & Weatherhead (2000) and Keller & Heske (2001) discovered adults and larvae of a burying beetle, *Nicrophorus pustulatus* Herschel, in a number of snake nests and hypothesized that a host shift had occurred. Instead of breeding solely on small vertebrate carrion, as do all known *Nicrophorus* spp., they suggest that *N. pustulatus* is the only animal that is a parasitoid of a vertebrate. Parasitoids are relatively large with respect to their host, only parasitic as larvae, live only within a single host, and eventually kill their hosts (Noble & Noble, 1971). The present study examines the responses of *N. pustulatus* and congeners to carrion, snake eggs and turtle eggs to evaluate Blouin-Demers and Weatherhead's hypothesis of a host shift, investigate cues used to identify appropriate resources and explore the mechanisms that might have allowed this host shift to evolve.

Biology of burying beetles

The typical burying beetle life history is well understood (Pukowski, 1933; Eggert & Müller, 1997; Scott, 1998). Adult burying beetles use environmental cues, principally odour, to locate resources at three critical stages. Cues are used in flight to find feeding resources when energetic reserves are low. Well-fed, reproductively ready beetles switch to a second set of cues to search for a breeding resource while in flight. For example, *Nicrophorus tomentosus* Weber adults are trapped exclusively at well-rotted carrion when they first emerge to feed in northern Michigan (June–July) but are attracted to small, fresh carcasses (a rare, ephemeral resource) during its breeding season (August–September) (Wilson *et al.*, 1984; Wilson & Knollenberg, 1984). Once a breeding resource has been approximately located, a beetle on the ground will orient using a third set of cues. With antennae spread and the head moving side to side, the beetle walks toward the resource using odour cues (Heinzel & Bohm, 1989; Boehm, 1995). Once on the carcass, the beetle assesses it by tasting, encircling and lifting (Trumbo *et al.*, 1995).

Females have a rapid juvenile hormone (JH) (Trumbo *et al.*, 1995) and ovarian (Scott & Traniello, 1987) response to discovering a carcass and eggs are oviposited in the soil in as little as 12 h. The carcass is buried, rounded and its hair or feathers removed. Larvae hatch several days later and crawl to the prepared carcass where they receive care from their parent(s). Parents regulate the size of the brood by first laying fewer eggs on smaller carcasses (Müller *et al.*, 1990b) and then by cannibalizing first instar larvae if the brood is too large for the resource (Bartlett, 1987; Trumbo, 1990a; Suzuki, 2004). If a male but no female discovers a carcass, the male will emit sex pheromone to attract a female (Pukowski, 1933; Eggert & Müller, 1989).

Other than its range, nocturnality, habitat requirements and phenology, the breeding biology of *N. pustulatus* has been enigmatic (Anderson & Peck, 1985; Peck & Kaulbars, 1987). *Nicrophorus pustulatus* has been caught in small numbers (0–6% of *Nicrophorus* specimens) at pitfall traps baited with well-rotted carrion, a typical *Nicrophorus* feeding response (Anderson, 1982; Scott & Traniello, 1990; Lomolino & Creighton, 1996; Ratcliffe, 1996; Backlund & Marrone, 1997; Bishop *et al.*, 2002 and references therein). Unlike other species of *Nicrophorus*, however, this species has never been caught on a fresh small carcass despite over 3000 trap-nights at sites located in Michigan, New Hampshire, North Carolina, Connecticut and Florida, USA (Wilson & Fudge, 1984; Wilson *et al.*, 1984; Scott *et al.*, 1987; Scott & Traniello, 1990; Trumbo & Bloch, 2000, 2002). *Nicrophorus pustulatus* successfully competes for, breeds on and provides parental care using a small, fresh carcass in the laboratory (Robertson, 1992; Trumbo, 1994; Rauter & Moore, 2002a, b; Trumbo & Valletta, 2007). The recent discovery of breeding adults of *N. pustulatus* in nests of two species of snakes (*Elaphe obsoleta* Say and *Elaphe vulpina* Baird & Girard) suggests that this species has undergone or is undergoing a remarkable change in reproductive biology.

Aims of the study

Blouin-Demers and Weatherhead's hypothesis of a host shift was evaluated in four ways. (1) *Nicrophorus orbicollis* Say and *Nicrophorus defodiens* Mannerheim were provided with snake eggs to determine whether use of snake eggs by *N. pustulatus* is a unique *Nicrophorus* behaviour requiring a host shift explanation. (2) Reproduction of *N. pustulatus* on snake and turtle eggs was compared to determine the specificity of cues that can elicit typical breeding behaviour on an alternative resource (vertebrate eggs). (3) *Nicrophorus pustulatus*' responses to carrion and snake eggs were

compared (choice of resource, preparation of resource, pheromone emission and JH changes) to investigate: (a) whether the two resources are differentiated, indicating selection to adaptively exploit the new resource; and (b) whether ancestral responses to carrion have been retained. (4) The literature was reviewed to ascertain whether previously anomalous aspects of the biology of *N. pustulatus* might be explained by a host shift.

Methods

General methods

A colony of *N. pustulatus* was established from wild-caught beetles collected in the research forest of Berea College, KY, USA. Beetles were maintained in containers ($17 \times 31 \times 11$ cm³) in groups of eight to 12 and kept at 20–22 °C and 15L : 9D and fed scraps of chicken liver. All experimental adult subjects were reared as larvae on mouse carcasses prepared by their parents. Prior to an experiment, individual adult beetles (25–40 days of age) were isolated for 24 h in a small container (9 cm diameter) with water but no food. Mouse carcasses (*Mus musculus* L.) were frozen shortly after death and thawed 24 h prior to experiments. Eggs of the brown house snake (*Lamprophis fuliginosus* Boie) were obtained from a snake breeding facility at the University of Texas at Tyler. Turtle eggs (*Chelydra serpentina* L. and *Chrysemys picta* Schneider) were obtained in the field from Kent, CT and Watertown, CT, USA.

Unless specified, experiments were conducted in covered $17 \times 31 \times 11$ cm³ breeding containers, half filled with soil, with a breeding resource covered by a paper towel. Larvae were produced in 10–15 days. Within 24 h of dispersing from a depleted resource, larvae were counted, washed and weighed. Because snake and turtle eggs were used as they became available, trials of these experiments were run over a 2-year period with different generations of *N. pustulatus*.

Use of snake eggs by *N. orbicollis* and *N. defodiens*

To determine whether *Nicrophorus* species other than *N. pustulatus* might use snake eggs as a breeding resource, 12 *N. orbicollis* pairs were provided two to three snake eggs (12.0–16.6 g) and eight *N. defodiens* pairs were provided one to two snake eggs (4.5–7.5 g). Breeding containers were checked daily and the snake eggs were visually inspected to make a qualitative assessment of their handling by beetles. If no larvae hatched and moved to the resource by day 10, the trial was terminated. *Nicrophorus orbicollis* were obtained from a laboratory colony started from beetles trapped in Bethany, CT, USA. *Nicrophorus defodiens* were wild-caught beetles trapped during their breeding season (late May to early June) in Kent, CT, USA.

Use of snake and turtle eggs and brood size regulation

To examine quantitatively the ability of *N. pustulatus* to use snake eggs as a breeding resource 14 male–female pairs were each provided one to five snake eggs (5.2–36.5 g). Similarly, 14 additional male–female pairs were each provided one to two turtle eggs (3.6–20.5 g). Containers were examined after 2 days to assess handling of the resource. Three dependent variables (number of larvae, total mass of the brood and mean mass of larvae) were regressed on resource mass (all r^2 values were adjusted). The criterion for brood size regulation was that the number of larvae would increase with resource size while the mean mass of larvae remained the same, over a significant portion of the resource size range. Efficiency was measured as total mass of the brood/total mass of resource. Coefficient of variation (standard deviation/mean) and Levene's test for equality of variances were used to compare variation in the efficiency of conversion of resource to beetle offspring for snake eggs vs. turtle eggs.

Movement and burial of carcasses and snake eggs

A rectangular box covered by a glass plate was used to compare movement and burial of snake eggs and carcasses (Fig. 1). Either two snake eggs ($n = 8$) or a mouse carcass ($n = 8$) of the same mass (±1 g) were placed in the box on top of thin soil and 25 cm away from a broad area of deep soil and leaf litter, suitable for burial. A male–female pair of *N. pustulatus* was placed under soil in one corner (lower left in Fig. 1) and was given 48 h to manipulate the resource.

Adult choice of carcasses and snake eggs

In an attempt to determine resource preference by *N. pustulatus* adults, male–female pairs ($n = 12$) were given a choice of two snake eggs or a mouse carcass. The eggs and carcass were placed at either end of a breeding container, approximately 20 cm apart. The size range was 11.4–15.9 g and the carcass and snake eggs were of equal mass (±0.2 g) for a particular trial. The container was inspected every 24 h until larvae dispersed from the nest.

Larval choice of carcasses and snake eggs

To examine preference by first instar larvae for a feeding resource, a snake egg (4.8–6.1 g) and chicken liver of equal mass (±0.2 g) were placed at either end of a breeding container, separated by 20 cm ($n = 8$). Ten *N. pustulatus* eggs, taken from separate breeding chambers, were placed halfway between the two resources. As the time of hatching neared, the container was checked at 24-h intervals and first instars on a resource were removed and their location recorded.
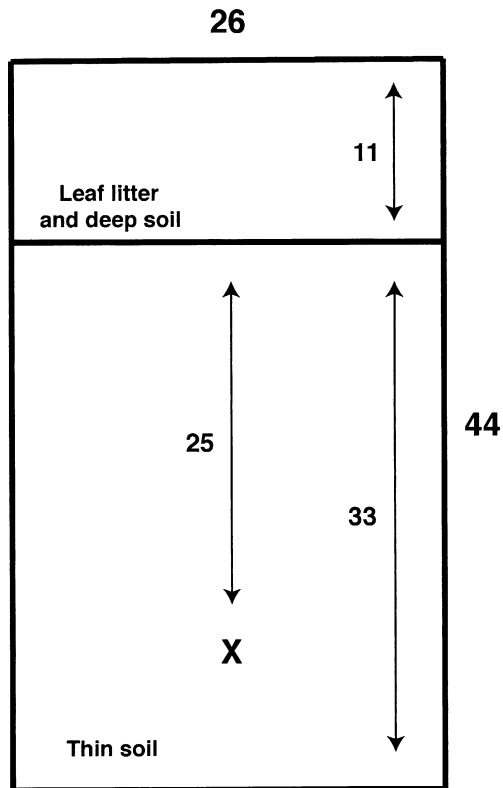
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Fig. 1 Set-up for investigating movement of a carcass or snake eggs by *Nicrophorus pustulatus*. The breeding resource was placed at the 'X' on thin soil (1.5-cm depth) unsuitable for burial, 25 cm away from deep soil (15-cm depth) and leaf litter. All measurements are in cm.

Pheromone emission

Pheromone emission by male *N. pustulatus* was compared in three contexts: without a resource, with a mouse carcass and with snake eggs. The apparatus was a glass terrarium ($30 \times 15 \times 20$ cm^3), one-half filled with soil and supplied with a rock perch for pheromone emission. Three males per treatment were observed on each of 30 June, 1 and 3 July 2005 (a total of nine males per treatment) from 20:30 to 24:00 hours under natural light, and scored every 30 min as releasing pheromone or not releasing pheromone based on posture (Eggert & Müller, 1989). Pheromone emission was scored from 0 (never releasing pheromone) to 8 (releasing pheromone at each observation time). Scores were converted to ranks and pair-wise comparisons were made using the Kruskal–Wallis test (SAS, 2000).

Hormonal response to carcasses and snake eggs

To examine the JH response to discovery of a breeding resource, females were provided with 12–15 g of mouse carcass ($n = 8$), 12–15 g of snake eggs ($n = 8$) or no resource ($n = 5$) during their active period. The resource was placed along the edge of a breeding container to facilitate discovery, and brief observations were made to confirm contact. Two hours after discovery, each female was quickly washed and dried, and a haemolymph sample (3.3–6.7 μL) was taken from severed hind legs with a calibrated microcapillary tube. The sample was immediately placed in chilled acetonitrile and kept at −70 °C. JH III titers were quantified by a radioimmunoassay (RIA). Extraction and RIA followed established methods for burying beetles (Trumbo *et al.*, 1995; Panaitof *et al.*, 2004; Scott & Panaitof, 2004) using the chiral-specific antiserum to 10R-JH III, the naturally occurring enantiomer (Hunnicutt *et al.*, 1989). JH III concentrations in the haemolymph were calculated from the volume of haemolymph taken, the extraction efficiency and its concentration in MeOH, and then multiplied by 0.5 as the standards were of racemic JH and the antibody is chiral specific. Because of the non-normal distribution of values for JH titer, the data were log transformed (SAS, 2000). Tukey–Kramer *t*-tests for multiple comparisons were used to determine statistically significant differences.

Results and discussion

Use of snake eggs by *N. orbicollis* and *N. defodiens*

Nicrophorus orbicollis pairs produced a brood in only one of 12 trials with snake eggs, and in that lone trial a single underweight larva dispersed from the nest. Except for this trial, the snake eggs remained white and showed little evidence of manipulation. We have noted that *N. orbicollis* will feed from opened but not from intact snake eggs (G. Smith & S.T. Trumbo, unpublished work). In none of the eight trials did *N. defodiens* rear a brood on snake eggs and eggs did not appear to have been closely inspected or manipulated.

Use of snake and turtle eggs and brood size regulation

Nicrophorus pustulatus pairs inspected and stayed near snake eggs, the female staying with larvae until larvae dispersed (Fig. 2a). Snake eggs became discoloured by beetle activity and by soil clumping where the parents opened the egg (within 24 h of discovery). In each trial ($n = 14$) larvae hatched and moved to the resource on day 4, the same timing that occurs with *N. pustulatus* using carcasses (Trumbo, 2006). The criteria for brood size regulation were met. There was a positive relationship between resource size and both number of larvae (Fig. 3, $F_{1,12} = 100.04$, $P < 0.0001$, $r^2 = 0.88$) and total brood mass ($F_{1,12} = 119.87$, $P < 0.0001$, $r^2 = 0.90$). The mean mass of larvae was not related to the size of the resource (Fig. 3, $F_{1,12} = 1.12$, $P = 0.31$, $r^2 = 0.01$). This
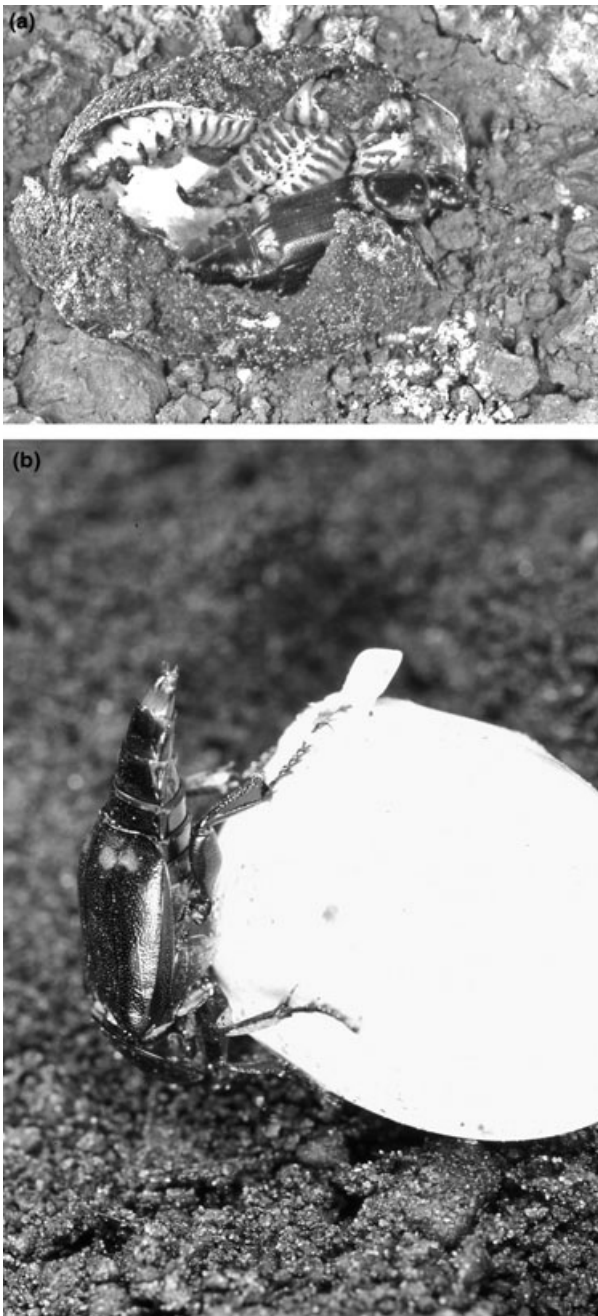
Fig. 2 (a) *Nicrophorus pustulatus* female with a small brood (five third instar larvae) on a single snake egg. (b) *N. pustulatus* male releasing pheromone from a snake egg.
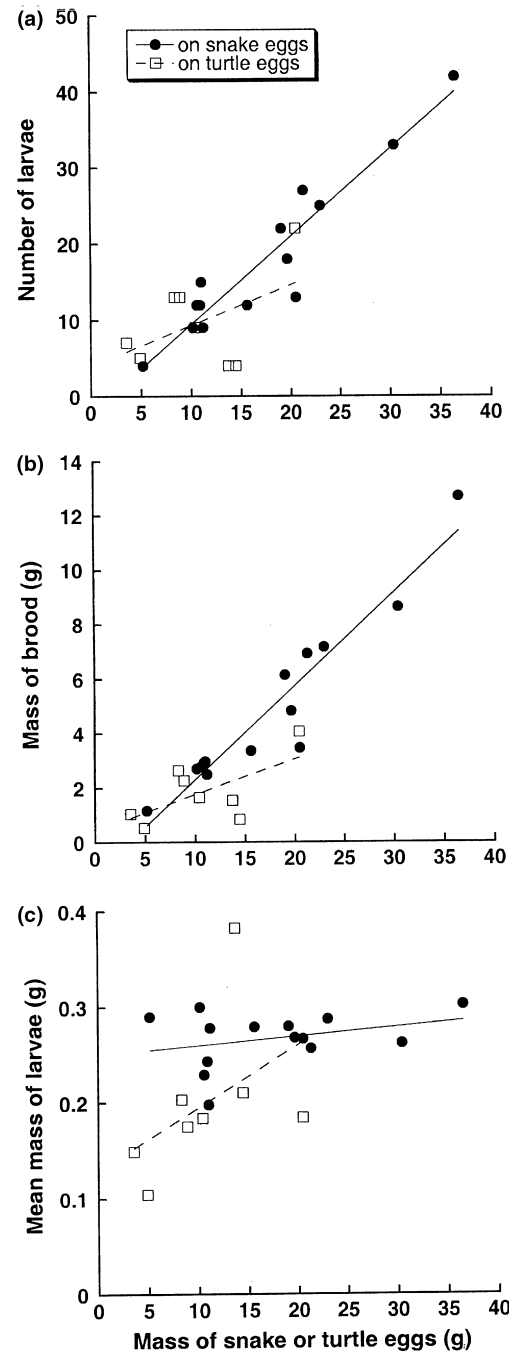

Fig. 3 The relationship of number of larvae (a), mass of brood (b) and mean mass of larvae (c) to mass of snake eggs (14 broods) or turtle eggs (eight broods).

is consistent with *Nicrophorus* use of carrion, where larval size is largely independent of the size of the carcass (Bartlett & Ashworth, 1988; Kozol *et al.*, 1988; Scott, 1990; Trumbo & Fernandez, 1995). Efficiency of conversion of snake egg mass to offspring mass was 26.6 ± 4.9% (mean ± SD; CV 0.184).

In eight of 14 trials, broods were produced with turtle eggs. This was a significantly lower proportion than with snake eggs ($P = 0.02$, Fisher's exact test). In three trials the larvae hatched and moved to the breeding resource on day 4 and in five trials the larvae arrived on day 6 or

after. In the eight trials with brood, the number of larvae (Fig. 3, $F_{1,6} = 1.80$, $P = 0.23$, $r^2 = 0.10$), the total brood mass ($F_{1,6} = 3.90$, $P = 0.09$, $r^2 = 0.29$) and mean mass of larvae ($F_{1,6} = 1.51$, $P = 0.26$, $r^2 = 0.07$) were unrelated to resource mass. There was low power to detect significant relationships when exploiting turtle eggs due to a smaller sample of broods, a smaller range of resource mass and larger variation in converting resource mass to beetle offspring. Although the conversion efficiency for two trials with turtle eggs was greater than the mean conversion efficiency for snake egg trials, overall efficiency was low ($18.7 \pm 9.3\%$, mean ± SD). Variance in efficiency when using turtle eggs was significantly greater than when using snake eggs (CV 0.497, Levene's test, $P < 0.02$).

The rapid and consistent use of snake eggs, the high degree of brood size regulation and the efficiency of conversion of resource mass to offspring mass [compare with the efficiency of carrion use by *Nicrophorus* spp. (range 18–28.5%) in Table 1 of Trumbo *et al.*, 2001], suggest that *N. pustulatus* is well adapted to using snake eggs for breeding. The weakness of each of these factors when exploiting turtle eggs suggests that although turtle eggs are a nutritionally acceptable substrate for larval development, *N. pustulatus* adults from Kentucky, USA, are not well adapted to respond to cues from this potential resource.

Movement and burial of carcasses and snake eggs

In each of eight trials with a mouse carcass, the *N. pustulatus* pair moved the carcass to the leaf litter and buried it within 48 h. Movement of a carcass to a suitable spot for burial was one of the earliest behaviours of this group to be studied (Fabre, 1899) and provided the name of this genus (*Nicrophorus* is a misspelled but nomenclaturally correct form of *Necrophorus*, literally 'carrier of the dead', Hatch, 1932; Herman, 1964). By contrast, snake eggs were never moved to the leaf litter ($P < 0.001$, Fisher's exact test), suggesting that burying behaviours are not stimulated by this resource. Snake eggs in the field are concealed by the ovipositing snake; concealment behaviour by *N. pustulatus* may be unnecessary.

Adult choice of carcasses and snake eggs

Broods were produced in 11 of 12 trials in which *N. pustulatus* pairs were provided a mouse carcass and snake eggs at opposite ends of a breeding container. Rather than making a choice, in 10 trials the mouse carcass was moved toward the snake eggs (never the snake eggs to the mouse, $P < 0.01$, binomial test), and the combined resources were used for feeding by larvae. In one trial, only the mouse carcass was used. Both resources were clearly recognized as appropriate for breeding, but the different responses to a carcass (trans-

port and hair removal) indicate that the two resources are differentiated.

Larval choice of carcasses and snake eggs

In all eight trials, first instar larvae were found only at the chicken liver and never at an intact snake egg ($P < 0.01$, binomial test) (a total of 75 larvae at chicken liver, five not recovered and 0 at snake eggs). This suggests that cues from the decaying liver were more attractive to larvae than any possible cue from snake eggs.

Pheromone emission

Pheromone emission occurred in all three contexts: without a resource (controls), with a mouse carcass and with snake eggs (Fig. 4). Pheromone emission was more frequent from mouse carcasses than from controls ($S = 110.5$, $P = 0.025$, Kruskal–Wallis test, pair-wise comparison) and more frequent from snake eggs than controls ($S = 113$, $P = 0.01$). Males emitted pheromone directly from snake eggs (Fig. 2b) without breaking through the shell, preventing degradation of the resource prior to breeding. The frequency of pheromone emission from mouse carcasses and snake eggs was not significantly different ($S = 95.5$, $P = 0.36$). Pheromone emission by males without a resource is an alternative mating strategy in burying beetles. The high frequency of male
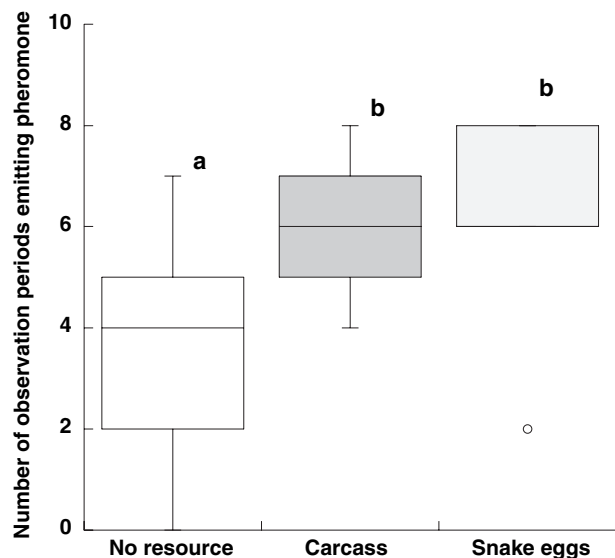
Fig. 4 Median, upper and lower quartiles and range of eight observation periods in which male *Nicrophorus pustulatus* were observed to emit pheromone. Different letters represent statistically significant differences in pheromone emission (Kruskal–Wallis test of ranks, SAS, 2000). The open circle represents an outlier that was included in the statistical analysis.

calling by pheromone release in the absence of a resource (46% of observation periods) suggests that females mate in the absence of larval food and that males obtain fertilizations from these matings, as occurs in congeners (Eggert & Müller, 1989). The higher rate of pheromone emission when either a carcass or snake eggs were present indicates that both resources stimulated male behaviour associated with an enhanced opportunity to breed and ensure paternity.

Hormonal response to carcasses and snake eggs

Two hours after exposure to a mouse carcass, JH titers in *N. pustulatus* females were elevated relative to either control females ($t = 2.97$, $P = 0.01$) or to females with snake eggs ($t = 2.33$, $P = 0.036$, Fig. 5). The trend toward a higher JH titer in females with snake eggs relative to controls was not significant ($t = 1.69$, $P = 0.12$, Fig. 5). JH titers in other species of *Nicrophorus* can be elevated in as little as 10 min after discovery of a carcass (Trumbo *et al.*, 1995). Changing levels of JH are thought to be important in organizing the breeding cycle in *Nicrophorus* (Scott *et al.*, 2001; Scott & Panaitof, 2004). The similar oviposition behaviour yet different JH profiles in response to mouse carcasses and snake eggs provide support for Panaitof & Scott's (2006) hypothesis that JH is not a gonadotropin in burying beetles.

General discussion

Blouin-Demers & Weatherhead (2000) hypothesized that *N. pustulatus* has undergone a host shift from carrion to
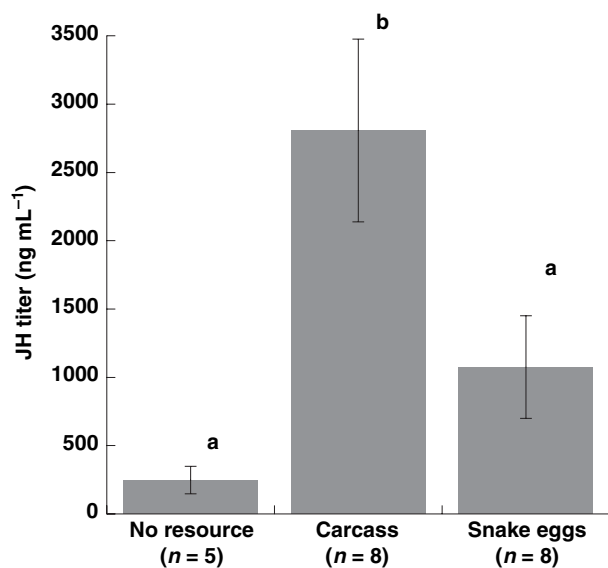

Fig. 5 Mean (±SE) titers of JH III, 2 h after discovery of a mouse carcass, snake eggs or no resource (control). Different letters represent statistically significant differences in JH titer (Tukey–Kramer *t*-test for multiple comparisons).

snake eggs and that the host shift was well established. Their primary evidence was the failure of past studies to find *N. pustulatus* at fresh carcasses, the presence of adult and larvae of *N. pustulatus* in snake nests and the large size of *N. pustulatus* clutches. The present study supports that hypothesis with four additional findings. (1) Congeners (*N. orbicollis* and *N. defodiens*) failed to respond to snake eggs as a breeding resource indicating that *N. pustulatus*' use of snake eggs is an evolved specialization. (2) *Nicrophorus pustulatus* exploited snake eggs efficiently, equalling the efficiency of *Nicrophorus*' use of carrion. (3) *Nicrophorus pustulatus* has a sophisticated ability to regulate brood size when using snake eggs. (4) *Nicrophorus pustulatus* treats snake eggs and carcasses differently and in adaptive ways. It is clear that neither resource is 'mistaken' for the other. *Nicrophorus pustulatus* has been found in nests of the black rat snake (*E. obsoleta*) and the fox snake (*E. vulpina*) (Blouin-Demers & Weatherhead, 2000). Its use of eggs of corn snakes (*Elaphe guttata* L.) (G. Smith & S.T. Trumbo, unpublished work) and brown house snakes in the present study suggest that *N. pustulatus* can exploit eggs of any oviparous snake that it can discover.

A host shift is often associated with a change in response to cues from potential hosts (Forbes *et al.*, 2005; Rossbach *et al.*, 2005; Olsson *et al.*, 2006). We hypothesize that the cues used by *N. pustulatus* in flight to locate feeding resources are largely unchanged from the ancestral state, that a new set of cues are used to locate resources for breeding while in flight, and that cues used on the ground to orient to a breeding resource have been broadened to include both carrion and reptile eggs. All species of *Nicrophorus* come to rotting carcasses to feed on carrion and carrion-feeding larvae. *Nicrophorus pustulatus* appears little different in this regard. Based on its absence on fresh carrion we suspect that flying *N. pustulatus* have lost the ability to respond to cues from fresh carrion. For most individuals in the field this would be sufficient to prevent the ancestral short-range response to carrion exhibited in the laboratory. The origin of the host shift from carrion to snake eggs may have been facilitated because unhatched or broken snake eggs emit odours of decay attractive to burying beetles (Blouin-Demers & Weatherhead, 2000). The cues presently employed to locate snake eggs are unknown.

An ecological benefit of a host shift is that interspecific competition can be avoided (Feder *et al.*, 1995; Messina, 2004b). This was probably important as *N. pustulatus* is the only known parasitoid of snake eggs, and interspecific competition for carrion is intense (Eggert & Müller, 1997; Scott, 1998). Use of snake eggs imposes a different challenge. Whereas there is no selection to minimize cues from putrefying carrion, eggs are designed to emit few odours, and what odours are emitted may be concealed by burial or other oviposition behaviours. Many insect parasitoids use general environmental cues to locate the approximate location of a host, and then use

host-specific cues at close range (Combes, 2005). *Nicrophorus pustulatus* appear to exploit larger, communal nests of *E. obsoleta* (black rat snake) more readily than smaller, single nests (Blouin-Demers *et al.*, 2004). Communal nests are not only larger in size but are often used in successive years (G. Blouin-Demers and P.J. Weatherhead, personal communication). The ability of *N. pustulatus* to locate a snake nest in a novel location (P.J. Weatherhead, personal communication) suggests that *N. pustulatus* does not rely solely on cues associated with old snake nests. The scarcity of *N. pustulatus* in small snake nests in the field is not caused by discovery and subsequent rejection of a resource that is too meagre. In the present study, breeding by females and pheromone emission by males was stimulated by a single snake egg of 5 g.

On the ground, *N. pustulatus* responds to either carrion or snake eggs in a manner that suggests that beetles distinguish between and adjust their manipulation of the two resources. A carcass but not snake eggs is moved and buried shallowly. It is likely that at some low frequency, *N. pustulatus* attempts to use carrion as a breeding resource in the field. *Nicrophorus pustulatus* will breed on mouse carcasses in the field if placed directly on the resource (S.T. Trumbo and G. Smith, unpublished work) and its efficiency in converting carrion mass to brood mass is equal to or greater than congeners (Trumbo *et al.*, 2001; Smith, 2005). A beetle might encounter a carcass before beginning a searching flight or after landing near a snake nest (in our laboratory a female used a dead neonate brown house snake (7 g) for breeding by curling it into a ball; S.T. Trumbo and G. Smith, unpublished work). The JH response by females and the increased pheromone emission by males in response to discovery of a carcass attest that the ancestral physiological and behavioural responses to carrion at short range are largely intact.

In the laboratory, larvae of *N. pustulatus* readily locate either a carcass or snake eggs. Odour from decaying meat may attract any nicrophorine larvae and may be the ancestral response. *Nicrophorus pustulatus* females will open a hole in a snake egg shortly after discovery. This hole will not only provide access to the resource but may provide cues of decay for larvae to locate the resource. *Nicrophorus* parents also use stridulations to attract first instar larvae to the nest (Niemitz & Krampe, 1972); this communication may be relatively more important for *N. pustulatus* than for other species of *Nicrophorus*. When selection on parents to choose an appropriate resource has been sufficiently strong, there may be little selection on offspring to discriminate among potential resources.

A number of anomalous aspects of the biology of *N. pustulatus* (its phoretic community, its large clutch size) need to be re-evaluated in light of the ability to use snake eggs for breeding. A host shift should affect phoretic organisms associated with burying beetles. *Poecilochirus carabi* Canestrini is a well-studied phoretic mite that reproduces on carrion using burying beetles as transport (Springett, 1968; Wilson & Knollenberg, 1987). Of five species of burying beetle studied in northern Michigan, *N. pustulatus* was the only one not carrying *Poecilochirus* (Wilson, 1982), suggesting that *Poecilochirus* cannot reliably find carrion using *N. pustulatus* as a carrier. *Nicrophorus pustulatus* produces the largest clutches, by far, among *Nicrophorus* spp. (200 vs. a range of 30–75) (Trumbo, 1992). A snake nest, especially a communal nest as is common in *E. obsoleta* (black rat snake), can contain a substantial mass of resource (Blouin-Demers & Weatherhead, 2000). Unlike larger carrion, an increase in snake egg mass does not entail a substantial increase in difficulty controlling decay or interspecific competitors. The large clutch size of *N. pustulatus* may permit superior exploitation of nests with a considerable mass of snake eggs and of snake clutches laid sequentially in communal nests.

Before the discovery of *N. pustulatus* in snake nests, the absence of this species on small carcasses and its large clutch size led to speculation that *N. pustulatus* may be a brood parasite (Trumbo, 1992) or a specialist on large carcasses (Trumbo, 1990b; Robertson, 1992). In a field study designed to examine both possibilities, *N. pustulatus* never was attracted to large, fresh carcasses, and the only case of brood parasitism was *N. tomentosus* parasitizing a brood of *N. orbicollis* (Trumbo, 1992). *Nicrophorus pustulatus* can parasitize other species of *Nicrophorus* in the laboratory, but this is now known to be common both intra- and interspecifically within this genus (Müller *et al.*, 1990a; Trumbo, 1994; Scott, 1997; Eggert & Müller, 2000; Suzuki, 2000), indicating that this is not unique to *N. pustulatus*. Larger carcasses (> 75 g) are known to be exploited by larger bodied species of burying beetles (Kozol *et al.*, 1988; Trumbo *et al.*, 2001); the moderate body size of *N. pustulatus* (Ratcliffe, 1996) is not consistent with the use of large carrion.

We hypothesize that the use of snake eggs was facilitated because a minimal number of new behaviours were required to evolve. The critical change was to recognize snake eggs as a potential resource. In particular, the switch from responding to feeding cues to responding to breeding cues, already present in adult *Nicrophorus* (Wilson & Knollenberg, 1984), seems to have been co-opted by *N. pustulatus* to employ a new set of cues for locating a resource for breeding. Several behaviours associated with the use of carrion are retained for use of snake eggs (oviposition near the resource, brood size regulation, increased pheromone emission, creating a hole in the resource for larval access and parental attendance). Many sophisticated resource preparation behaviours for carrion are not needed for using snake eggs and were dropped from the repertoire (removing hair, rounding and burial). That these behaviours can be elicited in response to carrion suggests that either carrion is sometimes used as a resource for breeding or behaviour associated with the host shift is still evolving.

An insect parasitoid of vertebrate eggs has conservation implications. *Nicrophorus pustulatus* has the potential to exploit any oviparous snake and perhaps other oviparous reptiles when sufficient cues can be obtained to locate a nest. Which vertebrates are being exploited will depend on the geographic range, habitat requirements and phenology of *N. pustulatus*, what cues are used to locate reptile nests, and whether additional species of *Nicrophorus* engage in similar behaviour. *Nicrophorus pustulatus* overlaps many species of oviparous snakes including one on the US federal list of threatened species (Indigo snake, *Drymarchon corais* Holbrook), and more than a dozen listed as endangered or threatened at the state level. *Nicrophorus pustulatus* also overlaps four species of oviparous snakes listed as endangered or threatened in Canada (*E. obsoleta*, *Elaphe gloydi* Conant, *Coluber constrictor foxii* Baird & Girard, *Heterodon platirhinos* Latreille). The lack of a response to turtle eggs in some trials and the delayed or variable response in other trials suggest that cues from turtle eggs are not as stimulating at short range as cues from snake eggs. The high efficiency with which a number of *N. pustulatus* pairs exploited turtle eggs, however, indicates that turtle eggs satisfy all the nutritional requirements for larvae. Because the geographic range of *N. pustulatus* extends somewhat north of the range of oviparous snakes, Blouin-Demers & Weatherhead (2000) speculated that turtles might be exploited in these areas. Although the two species of turtles used in this study place their nests underground and cover them with packed soil, there are a number of turtle species that place their nests in more accessible locations (Martof *et al.*, 1980). Nests of these species should be surveyed for *N. pustulatus* activity, and *N. pustulatus* from northern locations should be tested to determine whether they exhibit a more consistent response to turtle eggs.

Nicrophorus defodiens and *N. orbicollis* do not recognize the potential resource contained within an intact reptile egg. The latter species is only distantly related to *N. pustulatus*. *Nicrophorus defodiens* is often in a clade immediately basal to a larger group including *N. pustulatus*, the *marginatus* and *investigator* groups, thereby placing it relatively close to *N. pustulatus*. The phylogenetic placement of *N. pustulatus*, like the life history, has been problematic. Taxonomists have failed to reach a consensus on its phylogenetic history, using both precladistic (Portevin, 1926; Semenov-Tian-Shanskij & Medvedev, 1926; Hatch, 1927) and modern methods (Peck & Anderson, 1985). The most current analyses (Sikes & Peck, 2000; Sikes, 2003) suggested that *N. pustulatus* may be closely related to basal members of the *investigator* group of species, including the ecologically unstudied Hispaniolan endemic, *Nicrophorus hispaniola* Sikes and Peck, and the north-east Asian species, *Nicrophorus ussuriensis* Portevin. Given how poorly understood these close relatives are, we cannot specify at what point in evolutionary time this host shift may have occurred. It may have been an anagenetic change within the

N. pustulatus lineage itself or it may have preceded the origin of this species. To confidently determine when this host shift arose, the response of *N. hispaniola* and *N. ussuriensis* to snake eggs will need to be studied.

Conclusion

Behavioural and ecological studies suggest that *N. pustulatus* has undergone a host shift from carrion to snake eggs. Behavioural and physiological studies suggest that these two resources are differentiated; this differentiation may have been a critical adaptation in the transition from primary use of carrion to primary use of snake eggs. Although *N. pustulatus* may be able to exploit eggs of any oviparous snake in the laboratory, it probably has difficulty locating nests of some species in the field. Surveys of reptile nests as well as work on the sensory capabilities of *N. pustulatus* are necessary to understand the breadth of this host shift to a parasitoid ecology.

Acknowledgments

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