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Parental care improves offspring survival and growth in burying beetles

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Abstract. Burying beetles (genus *Nicrophorus*) provide elaborate parental care to their offspring. Parental beetles defend a small vertebrate carcass, which constitutes the sole food source for the larvae. They also manipulate the carcass in various ways and directly regurgitate pre-digested carrion to the young. The benefits of carcass manipulation and regurgitation have been the subject of a few small-scale studies that have yielded conflicting results. In this study, we investigated the benefits of these behaviours and tested for possible beneficial effects on larval survival rates and final body mass in *N. vespilloides*. In this species: (1) larval survival and mass were significantly higher in broods receiving parental care throughout larval development on the carcass than in broods developing in the absence of adults; (2) parental presence immediately subsequent to larval hatching greatly improved larval survival rates; (3) continued parental presence for several days further improved larval growth, leading to a greater final mass of individual larvae; (4) larval survival and growth were improved by parental preparation of carcasses and by an excision made in the integument of the carcass surface by the parents that allows the larvae ready access to their food; (5) positive effects of parental feeding on larval survival and growth were not mediated by the transfer of symbionts.

Costs and benefits associated with parental investment have received much attention from students of behavioural ecology in the last two decades (reviewed in Clutton-Brock & Godfray 1991). In many species, parents invest time and energy in rearing their offspring, and they take risks in defending their young against predators, resulting in reduced opportunities for future reproduction. Improved offspring survival or quality, or, in the case of male parental care, improved mating or fertilization success must offset these costs to maintain the parental investment pattern. In many species with parental care, most of them vertebrates, substantial benefits to young have been demonstrated (reviewed in Clutton-Brock 1991). Most young birds and mammals, for example, remain heavily dependent on parental provisioning or defence for extended periods. Among invertebrates, however, parental care is relatively infrequent. Only a few studies have investigated its benefits, focusing mostly on the guarding of eggs and young (e.g. Tallamy & Denno 1981); for most care-giving invertebrates, such studies are lacking or incomplete, including one of the better-studied examples, the genus *Nicrophorus* (burying beetles).

In all *Nicrophorus* species studied to date, adult beetles bury small vertebrate carcasses, shape them into a ball and later regurgitate predigested carrion to the young larvae on the carcass (Pukowski 1933; Bartlett 1988; Fetherston et al. 1990; Robertson 1992). The adults remain with the brood for several days, defending the larvae and the carcass against predators and other carrion feeders. Males of all species studied participate in parental care, and like females, they also make competent single parents: under laboratory conditions, a male whose mate disappears from the carcass can raise a brood as successfully as a single female or a pair (Bartlett 1988; Reinking 1988; Trumbo 1991). Experimental studies of biparental care have not detected any benefits

Dedicated to Prof. Dr. G. Osche on the occasion of his 70th birthday.

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arising from biparental feeding in the laboratory (Bartlett 1988; Scott 1989; Trumbo 1991), but have shown that biparental care may lower the risk of brood failure in the field when the density of congeneric competitors is high (Trumbo 1990a, b, 1991; Scott 1994). Brood failures can occur when superior congeneric competitors (intra- or interspecific) detect the buried carcass and destroy the brood present on the carcass prior to establishing their own brood (Scott 1990, 1994; Trumbo 1990a, b).

The benefits of parental care have been peripherally addressed in a few studies (Pukowski 1933; Wilson & Fudge 1984; Trumbo 1992), which yielded conflicting results as to the extent to which young benefit from parental regurgitation and carcass maintenance. To resolve the issue for at least one species, we carried out a series of experiments on the most abundant species in Central Europe, *N. vespilloides*. We addressed the following questions. (1) To what extent do different phases of the period of parental care contribute to offspring survival? These phases include a prehatching period, during which parents prepare the carcass and the female oviposits, and a posthatching period, during which parents directly interact with their offspring. (2) How important is the transfer of symbionts from parents to offspring to larval survival and growth? Regurgitation and parental preparation of larval food are characteristic of many insects that feed on cellulose-rich substrates and require the transfer of symbiotic protozoa, bacteria or fungi to inoculate the larval intestine or the substrate itself with these symbionts (several families of termites (Isoptera); the woodroach, *Cryptocercus punctulatus* (Dictyoptera): Nalepa 1984; ambrosia beetles, *Austroplatypus incompertus* (Curculionidae): Kent & Simpson 1992; and various species of passalid beetles (Passalidae): Schuster & Schuster 1997). Despite the vastly different nature of the larval food source in burying beetles, symbiotic bacteria or other beneficial micro-organisms could be transferred during regurgitation. We addressed this possibility in our final experiment.

GENERAL METHODS

Origin and Maintenance of Experimental Animals

We collected beetles from the field in carrion-baited pitfall traps. Our trapping site was located in Northern Germany near the town of Bielefeld, in a deciduous forest (52°01′N, 8°23′E). Unless otherwise stated, experimental animals were first-generation offspring of field-caught beetles between 20 and 50 days adult age. All of the animals used (except in the symbiont experiment) had been reared by foster parents from laboratory cultures and were therefore free of the nematodes or mites that are typically found on field-caught beetles. (Our method of establishing a laboratory culture of foster parents is described in experiment 4 for the rearing of symbiont-free beetles.) We maintained all beetles in temperature-controlled chambers at 20°C under a 16:8 h light:dark cycle. Groups of up to six adults of the same sex were kept in small transparent plastic containers (10 × 10 × 7 cm) with moist peat, and were fed dead mealworms twice a week.

Experimental Design

Reproduction is easily induced by providing sexually mature beetles with a suitable carcass. For carcasses, we used laboratory mice that had been frozen fresh and re-thawed prior to the experiment. At a time when beetles are typically searching for carrion (4 h before lights off), we placed male–female pairs in clean transparent containers (10 × 10 × 7 cm) two-thirds filled with peat, and provided them with a mouse carcass. Containers were stored in the temperature-controlled chambers described above and transferred to a dark chamber at 20°C upon carcass burial. At this temperature, female *N. vespilloides* typically begin to oviposit 8–24 h after their first contact with the carcass (A. K. Eggert, M. Reinking & J. K. Müller, unpublished data), and eggs take a mean of 56 h to develop (Müller & Eggert 1990). We transferred the beetles and the carcass to a clean container with peat 40 h after they had received a carcass. Transfers occurred under dim red light to minimize disturbance to beetles. We then searched the previous container for eggs using flexible forceps, and any eggs found were stored on moist filter paper at 20°C until the larvae hatched. Eggs were checked every 8 h, because first-instar larvae do not survive much longer than this without food.

The adult beetles on the carcass received larvae as soon as their own larvae had begun to hatch. Larvae encountered before this time are frequently killed and eaten by adult females

(Müller & Eggert 1990). Before adding larvae, the adults and the carcass were always transferred to a new container, so that larvae hatching from eggs that might have been present in the old container could not confound our results. Once broods had been established, replicates were checked at least twice a day to see whether the parent had abandoned the brood. Parents that have decided to desert the brood typically run around on top of the peat and can be seen easily during checks. When this occurred, we removed the parent. When the larvae dispersed from the remains of the carcass, they were collected from the peat, counted and weighed individually to the nearest mg.

Carcass mass (15.0 ± 0.3 g) and the number of first-instar larvae added (15) were kept constant for all experiments to avoid the confounding effects of variation in egg clutch size (Müller et al. 1990a). When larger numbers of larvae appear on a 15-g carcass, parents regularly practice infanticidal brood reduction (Bartlett 1987); with the carcass size and larval numbers used here, infanticide is rare (Bartlett 1987). The larvae placed on a carcass with a caring adult were not necessarily the caring adult's own offspring. Larvae from other broods could be used because the beetles do not discriminate between their own and unrelated offspring except on the basis of temporal cues (Müller & Eggert 1990).

Statistics

We first tested data for normality using Shapiro–Wilks W before proceeding with further analyses. We analysed the number of surviving larvae using non-parametric statistics because this number deviated significantly from normality in all experimental groups (all $P<0.01$) except one (broods that received 12 h of post-hatching care; $0.1<P<0.2$). We used parametric procedures for the analysis of the mean mass of surviving larvae, which showed deviations from normality in only one experimental group (broods that received 12 h of post-hatching care; $P<0.01$; all other $P>0.05$). For our analyses, we used the mean larval mass from each brood rather than individual larval masses. For pair-wise comparisons in experiments with three or more treatment groups (Figs 1–3), we used the Tukey–Kramer procedure for ranked data (Zar 1984) for the number of surviving larvae, and the Tukey–Kramer procedure for parametric data for the mean mass of surviving larvae.

We used the JMP statistical package for Macintosh computers; for tests not available in this package, we used formulae given in Zar (1984). When no larvae survived, the respective value for the number of larvae surviving to dispersal was zero, and the mean weight of surviving larvae was treated as a missing value.

EXPERIMENT 1: BENEFITS OF PRE- AND POST-HATCHING PARENTAL CARE (CARCASS PREPARATION AND REGURGITATION)

Methods

In addition to defence of the carcass and the brood, parental care in burying beetles involves two components: (1) pre-hatching care, which involves interring the carcass, rolling it into a ball, removing fur and fungi from the carrion ball, spreading anal secretions over its surface, and possibly pre-digesting the carrion by regurgitating digestive secretions (Pukowski 1933); and (2) post-hatching care, which encompasses the creation of an opening in the carcass, direct regurgitation of pre-digested carrion to larvae after the latter have reached the carcass, and continued carcass maintenance. To assess the relative importance of these activities for larval development, we placed groups of 15 first-instar larvae by themselves either on a fresh carcass (no parental care, $N=42$), or on a carcass that had been prepared by beetles (pre-hatching care only, $N=21$), or on a carcass on which both parents were present (pre- and post-hatching care, $N=34$). For the first group (no care), we used fresh carcasses that had not been manipulated by beetles but that had a hole cut in the abdomen to facilitate larval access to the carcass; for the second group (pre-hatching care), we used carcasses that had been buried and treated by a pair of beetles until their first larva hatched. When the surviving larvae left the carcass, we counted and weighed them. The general experimental design was as described above.

Results

Experimental conditions significantly affected the number of larvae surviving to dispersal (Kruskal–Wallis test: $H_2=38.64$, $P<0.0001$; Fig. 1a) as well as mean larval mass (ANOVA,

$F_{2,77}=144.5$, $P<0.0001$; Fig. 1b). However, this effect was due solely to significant differences between broods that received post-hatching care and broods that did not. Larval survival and the body mass of surviving larvae were not significantly different between the treatments without parental care and the one with pre-hatching care only (Fig. 1).

EXPERIMENT 2: PHASES OF POST-HATCHING PARENTAL CARE

Methods

To assess the relevance of different phases of post-hatching care, we manipulated the duration of parental care by experimentally terminating the presence of parents on the carcass. We compared the success of broods that had received 0 ($N=21$), 12 ($N=20$), 24 ($N=20$), 48 ($N=22$) or 120 h ($N=28$) of post-hatching maternal care; 120 h is equivalent to the normal duration of parental care under the given laboratory conditions. Post-hatching care in all groups started when larvae were placed on the carcass and was terminated by removing the caring female after the prescribed period of care. Data for the 0-h group were taken from broods that experienced pre-hatching care only in experiment 1.

Results

The duration of care had significant effects on the number of larvae surviving (Spearman rank correlation: $r_s=0.485$, $N=111$, $P<0.0001$; Fig. 2a) and on the mean body mass of surviving larvae (linear regression: $r^2=0.322$, $N=100$, $P<0.0001$; Fig. 2b).

Subsequent multiple comparisons revealed that the effect of the duration of parental care on larval survival was due to significant differences between broods without parental care (0 h) and the remaining groups. Survival rates were much lower in broods without care than in broods that received care (Fig. 2a). Survival rates were similar among the groups that received parental care for at least 12 h (12, 24, 48 or 120 h; Fig. 2a).

The body mass of surviving larvae showed a slightly different pattern (Fig. 2b). Multiple comparisons between the treatment groups revealed that larvae from broods without parental care had a lower body mass at the time of dispersal than did larvae in the groups that received parental care. The mean body mass of dispersing larvae continued to increase, however, with increasing durations of parental care beyond the first 12 h. Larvae from broods with 48 h or 120 h of care were significantly heavier at dispersal than larvae receiving 12 h of care, and larvae from broods with 120 h of care were significantly heavier than larvae from broods with 24 h of care.

These results suggest that parental care continues to have positive effects on larval growth throughout the first 48 h of larval development.

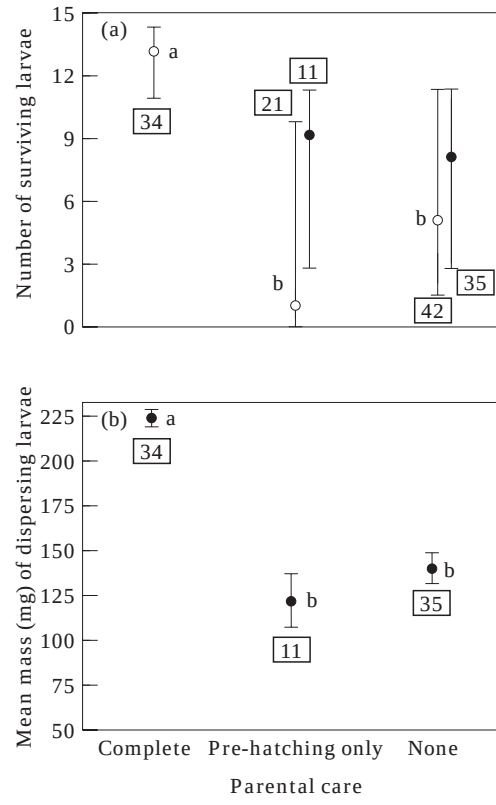

Figure 1. (a) Number of larvae surviving in broods of 15 (median and inter-quartile range); (b) mean ± SE mass of surviving larvae at dispersal in successful broods under conditions of full-length parental care, pre-hatching care only and no parental care. In (a) values for successful broods only (●) are shown in addition to the complete sample (○) whenever brood failures occurred in a treatment group. Different letters denote significant differences between the different treatment groups ($P<0.05$). Numbers in boxes are sample sizes.

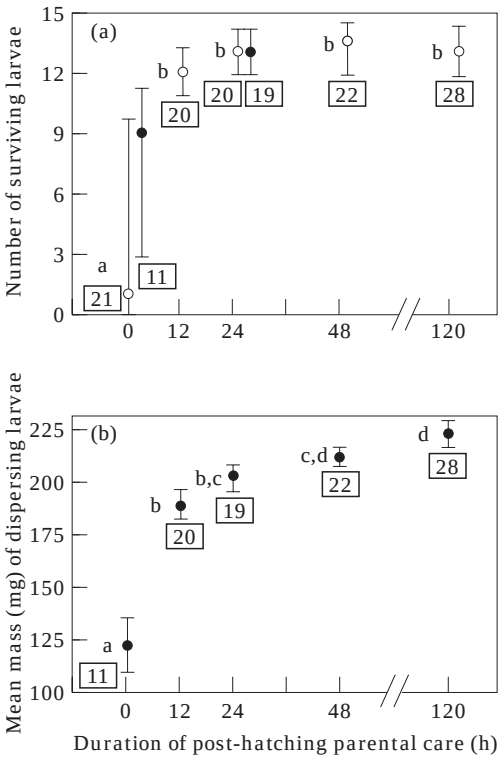

Figure 2. (a) Number of larvae surviving in broods of 15 (median and inter-quartile range); (b) mean ± SE mass of surviving larvae at dispersal in successful broods receiving parental care for different periods of time after hatching. In (a) values for successful broods only (●) are shown in addition to the complete sample (○) whenever brood failures occurred in a treatment group. Different letters denote significant differences between treatment groups ($P<0.05$). Numbers in boxes are sample sizes.

The mean body mass of larvae that received 48 h of care did not differ significantly from the body mass of larvae that received full-length maternal care (120 h; Fig. 2b). This result may indicate that the last 72 h of care have no effect on larval growth, or that the effect is too small to be significant, given the degree of variation and our sample sizes.

EXPERIMENT 3: OPENING THE CARCASS

Methods

At about the time the first larvae appear on the carcass, the parents chew a hole in the skin of the carcass. First-instar larvae assemble in this opening to feed and be fed; parental and larval feeding enlarges this opening and eventually leads to a hollowing-out of the carcass from the inside. To assess the importance of the existence of the small initial opening to larvae arriving on the carcass, we compared the survival and final mass of larvae without post-hatching care on prepared and unprepared carcasses with and without a hole. All carcasses had been frozen prior to the experiment and were thawed when larvae hatched.

Prepared carcasses were those that had been prepared by a pair of beetles until their own larvae hatched. To obtain prepared carcasses without an opening ($N=18$), we removed the carcass before parental beetles started to feed larvae. Carcasses that already had a small hole at this time, as occurs occasionally, were not used. To obtain prepared carcasses with an opening ($N=22$), parental beetles were permitted to feed their own first-instar larvae for 12 h; after this time, carcasses always had a conspicuous opening with a diameter of approximately 0.5 cm.

Unprepared carcasses were those that had not previously been manipulated by beetles. For unprepared carcasses with an opening ($N=15$), we used scissors to cut a hole of about 1 cm^2 into the skin on the thigh of a hind leg; unprepared carcasses without a hole ($N=22$) remained unmanipulated.

Results

Our treatments had clear effects on the number of larvae surviving to dispersal ($H_3=33.62$, $P<0.0001$; Fig. 3a), and on the body mass of surviving larvae ($F_{3,62}=11.84$, $P<0.0001$; Fig. 3b). Multiple comparisons of the number of larvae surviving in different groups (Fig. 3a) revealed that more larvae survived on unprepared and prepared carcasses if they had an opening. Significantly fewer larvae survived on unprepared carcasses without an opening than on prepared carcasses with an opening. On prepared carcasses with an opening, the number of larvae surviving to dispersal was very high, and similar to values observed in broods receiving post-hatching parental care (e.g. Figs 1a and 2a). Thus, preparation of the carcass, and even more so the presence of an opening in the carcass, apparently improves larval survival. For mean larval weights, an ANOVA allowed a separation of factors involved in the

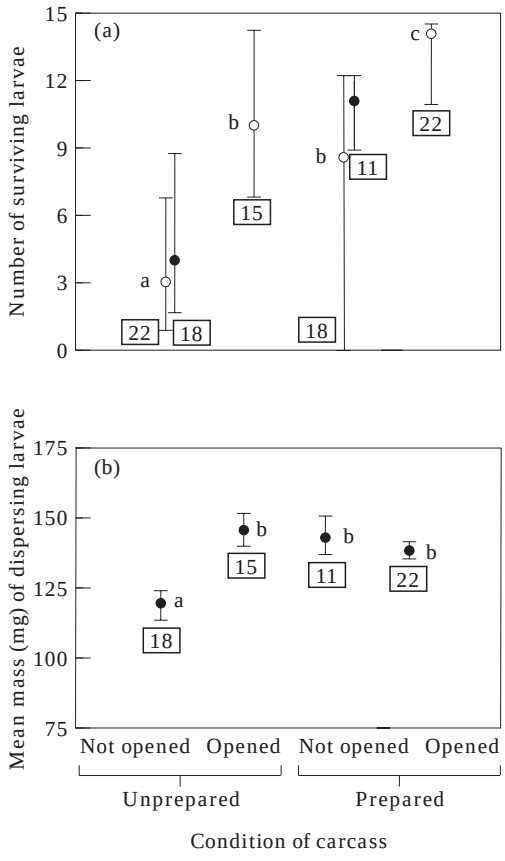

Figure 3. (a) Number of larvae surviving in broods of 15 (median and inter-quartile range); (b) mean ± SE mass of larvae surviving to dispersal, in broods receiving no post-hatching parental care on different types of carcasses. In (a) values for successful broods only (●) are shown in addition to the complete sample (○) whenever brood failures occurred in a treatment group. Different letters denote significant differences between complete samples ($P<0.05$). Numbers in boxes are sample sizes.

overall effect; there were significant effects of carcass preparation by adult beetles ($F_{1,62}=5.19$, $P=0.026$) and of the presence or absence of an opening in the carcass ($F_{1,62}=8.48$, $P=0.005$); the interaction between these factors was also significant ($F_{1,62}=19.60$, $P<0.0001$). The existence of an opening did not affect larval weights equally on unprepared and prepared carcasses: mean larval masses were higher when the carcass was opened on unprepared carcasses but not on prepared carcasses.

EXPERIMENT 4: A TEST OF THE SYMBIONT-TRANSFER HYPOTHESIS

Methods

For this experiment, offspring from field-caught pairs were reared under one of two conditions. For the production of 'symbiont-carrying' beetles, we provided field-caught pairs with 20-g carcasses in the field and allowed them to rear their own offspring in undisturbed breeding attempts. We collected offspring upon their emergence from their pupal chambers in the soil.

To obtain symbiont-free 'orphaned' beetles, we provided field-caught pairs with a carcass and collected eggs from the peat to be stored on moist filter paper as described above. Larvae hatching from these eggs (15 per female) were not placed back on their parents' carcass; instead, they were provided with fresh 15-g carcasses to develop without any post-hatching care. If a hole is cut into the carcass surface, larval survival under these conditions is relatively high (Fig. 3a).

For a test of the symbiont-transfer hypothesis, both the symbiont-free and the symbiont-carrying individuals were then allowed to produce a second filial generation. We removed phoretic mites, *Poecilochirus carabi*, from symbiont-carrying beetles before we provided them with a carcass. In both the symbiont-free and in the symbiont-carrying trials, one adult provided care for 15 of his or her own larvae on a 15-g carcass; the other parent was removed from the brood at the time the first larva hatched. Any larvae present at dispersal were counted and weighed.

Results

If the symbiont-transfer hypothesis is upheld, 'symbiont-free' beetles, which could not have received symbionts from their parents, should be less successful than controls in providing parental care to their own young, resulting in reduced larval survival, lower larval weights at dispersal, or both. We found similar results, however, for 'symbiont-free' beetles and 'symbiont-carrying' controls (Table I). Broods raised by the two treatment groups contained the same number of larvae at the time larvae dispersed, and the dispersing larvae had the same mean mass in both groups.

Table I. Comparison of symbiont-free broods and symbiont-carrying controls with respect to the number of larvae surviving to dispersal and the mean mass of surviving larvae (initial brood size = 15 larvae)

Treatment	Symbiont-free ($N = 17$)	Control ($N = 29$)	Test
Mean number of larvae surviving to dispersal (1st and 3rd quartile)	14.0 (13.5–15.0)	15.0 (14.0–15.0)	Mann-Whitney U, $U = 267.5$, $P = 0.61$
Mean ± SE mass of surviving larvae (mg)	203.5 ± 3.9	204.5 ± 2.2	Student's t, $t = 0.242$, $P = 0.81$

DISCUSSION

Parental care in burying beetles involves at least two biologically significant components. Parents manipulate the larval food source in a way that maximizes larval survival and growth, including direct regurgitation of pre-digested carrion to the larvae, and they defend both larvae and carcass. Our study focused on the nutritional effects of parental food preparation and regurgitation and did not consider effects related to parental defence of carcass and brood. Carcass preparation and maintenance may reduce the emanation of odours attractive to competitors and thus have important consequences for carcass defence; our study did not consider such effects. The importance of parental defence, including reduced attraction of competitors, depends on the risk of losing the carcass or the brood and thus on the frequency with which competitors or predators locate the carcass. Repeated attempts at quantifying the benefits of parental defence in the field (Scott 1990, 1994; Trumbo 1990b) have revealed that such benefits are contingent on the competitive environment, particularly the abundance of congeneric competitors. The nutritional effects of parental food manipulation and regurgitation, in contrast, are much less likely to depend on environmental variables and thus lend themselves more easily to a controlled study in a laboratory setting.

Our results show that parental care in *N. vespilloides* has beneficial effects on larval survival and final mass even when brood defence is disregarded. Experiment 1 showed that direct parental care of larvae is very important for larval survival and growth, and it suggests that such care is more important than the preparation of the carcass prior to the hatching of larvae. For two reasons, however, the latter result needs to be interpreted with caution. First, the presence or absence of an opening in the carcass had significant effects on larval survival (Fig. 3a), and this was an uncontrolled confounding factor in experiment 1. When adult beetles are preparing a carcass, they may create such an opening either shortly before or shortly after the larvae come to the carcass. In our experiment, all the 'unprepared' carcasses, but only a few of the 'prepared' carcasses had an opening. This factor probably resulted in the higher failure rate on prepared carcasses (10/21 broods versus 7/42 on unprepared carcasses; log-likelihood ratio, $G_1 = 6.559$, $P = 0.010$), and concealed the effects of carcass preparation that became apparent in experiment 3.

Secondly, at the time larvae were added, 'unprepared' carcasses were fresh, while 'prepared' carcasses were already 64 to 80 hours old, making the interpretation of this experiment more difficult. Carcass manipulation by adult beetles may slow the decomposition process and help preserve carcass value, effects that would have been overlooked in our experiment. To resolve this issue, it would be necessary to add an experimental group of broods on aged, unprepared carcasses to those used in our experiment.

Different parts of the 5-day period of post-hatching care differ in their importance for larval development. The first 12 h have the most profound effects on larval survival and growth, presumably because parents at this time create an opening within which larvae assemble and feed. In the North American species, *N. orbicollis*, parental presence immediately after hatching also appears to be essential for larval survival (Sherwood & M. P. Scott, unpublished data, cited in Fetherston et al. 1990). When *N. vespilloides* parents stay for periods longer than 12 h, however, they still contribute significantly to the growth of larvae, but larval survival is already close to 100% and cannot increase further with longer care. In another laboratory study on *N. orbicollis* (Scott & Traniello 1990), larvae from broods receiving full-length maternal care

attained a greater mean mass than larvae from broods with roughly 48 h of maternal care, which supports our conclusion that parental regurgitations after 1 or 2 days after hatching continue to improve larval growth.

Our results also show that burying beetle young have much higher survival rates when an adult beetle makes the carcass accessible for the larvae by chewing an opening into the surface. The existence of an opening improved larval survival even on unprepared carcasses. The location of the opening may also affect larval survival; in experiment 3, unprepared opened carcasses had a hole in the thigh, and the number of surviving larvae was significantly greater than on unprepared carcasses in experiment 1, which had a hole in the abdomen (Mann–Whitney U-test: $U=470.5$, $Z=2.828$, $N_1=15$, $N_2=42$, $P=0.0047$). Digestive enzymes in the intestines of the carcass may negatively affect the survival of larvae that start feeding within the abdominal cavity. Adult beetles preparing fresh mouse carcasses frequently remove part of the intestine from the abdomen and re-seal the opening (J. K. Müller, personal observation).

Carcass preparation by adults also had independent positive effects on larval survival and, on unopened carcasses, on larval growth. The removal of fur and the spreading of oral and anal secretions on the carcass appear to make the carcass more readily accessible to the larvae and may facilitate their movements across the surface of the carrion ball. We did not address the possibility that carcass preparation may help preserve the carcass and decelerate its decomposition; if our 'unprepared' carcasses had been aged to resemble 'prepared' carcasses, the positive effects of carcass preparation might have been more pronounced.

Trumbo (1990c) also noted that larvae of *N. defodiens* sometimes have difficulty gaining access to the interior of a carcass when parents are absent. Successful 'orphaned' broods in our experiments seemed to be broods in which larvae jointly fed at one particular site on the carcass, suggesting that the joint production of digestive enzymes, or use of mandibles, may facilitate access to the carrion for such broods. Similarly, tough surfaces on plants can prevent phytophagous insect larvae from accessing their actual food substrate beneath, and in the chrysomelid beetle, *Phratora laticollis*, mortality in the first 2 days of first instars was higher in single larvae than in groups of 10 larvae (Grégoire 1988). The observed crowding of larvae around existing openings in the surface, however, need not indicate a truly cooperative effort; it may simply be due to single larvae joining their more successful peers. Fetherston et al. (1990) suggested that to survive without continued regurgitation, *N. orbicollis* larvae must have a few hours of contact with parents during which they are fed. Possibly, however, as in *N. vespilloides*, the first hours of contact with parents are important because at this time, the parents create an opening that allows access to the carrion. Alternatively, this result may indicate that parental regurgitation is less important for *N. vespilloides* than it is for *N. orbicollis* larvae (see also Trumbo 1992).

For *N. vespilloides*, endosymbiont transfer is not an important aspect of parental regurgitation. This species probably does not need endosymbionts for the digestion of their food resource or for the production of specific nutrients. Carrion is probably one of the most easily digested and protein-rich substrates available in nature (Tallamy & Wood 1986), and may therefore not require the presence of micro-organisms to provide a complete diet. Species in which symbiont transfer from parents to young is important, like woodroaches and termites, use wood as a food resource and depend on the primary digestion of cellulose by flagellates (Nalepa 1984). No information is available about possible endosymbionts of burying beetles. Although burying beetles may transfer micro-organisms to their young during regurgitation, such transfer is more likely to be casual and accidental than to serve a specific nutritional function.

Studies on the importance of parental regurgitation and carcass preparation have typically compared broods with full-length parental care to broods in which the parents were removed from the carcass between the initiation of oviposition and emergence of the first larva. In the earliest study of this kind (Pukowski 1933), probably on *N. vespillo*, the majority of 'orphaned' larvae matured to pupation even when the female was removed before the first act of regurgitation (89% survival in 14 broods with 147 larvae, compared to 100% of 89 larvae in eight broods receiving post-hatching parental care). Unlike any of the later studies, however, Pukowski observed a severe disadvantage to larvae without care after

pupation (only one of 33 pupae emerged as an adult beetle, compared to roughly 50% of pupae that had received care). In our own experiments on *N. vespilloides*, more than 90% of larvae survived to adulthood, suggesting that the high mortality in Pukowski's study was at least partly due to inadequate rearing conditions after larvae had left the carcass (e.g. inappropriate substrate, insufficient moisture). In our experiments, the only larvae that consistently experienced lower survival rates were those at the bottom of the body mass range (<40 mg body mass at the time of dispersal from the carcass).

Nicrophorus orbicollis broods receiving parental care produced young more frequently (96% of broods) than did broods without parental care (26%; Wilson & Fudge 1984). Where larvae survived, they were also fewer, and attained a lower body mass before dispersal, than larvae that received care. Differences in larval numbers between the two groups may have been partly due to the removal of eggs from the soil for counting, which occurred in the no-care group only. Handling of eggs can lead to significantly reduced hatching rates (Bartlett & Ashworth 1988).

When access to food was facilitated by offering a very soft substrate such as chopped liver, larvae of *N. defodiens*, *N. tomentosus* and *N. pustulatus* survived well even in the absence of parents (Trumbo 1990c, 1992), but *N. orbicollis* and *N. sayi* failed to reach even the second instar under the same conditions (Trumbo 1992). This result suggests that the latter two species depend on parental regurgitation even more than does *N. vespilloides*. In an unpublished laboratory study, we were able to raise *N. vespilloides* but not *N. orbicollis* larvae on chopped beef liver (J. K. Müller, unpublished data).

The proximate mechanisms that mediate the beneficial effects of parental regurgitations remain unknown. They apparently are not due to endosymbiont transfer, but otherwise they are little understood. Pukowski (1933) hypothesized that continued regurgitations ensure an adequate food supply for larvae immediately subsequent to hatching and larval moults, that is, when the larval mouthparts are still soft and incompletely sclerotized. Our results suggest that parental regurgitations continue to confer benefits beyond the moult to the third and last instar, which in *N. vespilloides* occurs approximately 38 h after a larva first reaches the carcass (at 20°C: Müller & Eggert 1990). Due to their larger size, parents may simply be capable of producing larger amounts of proteolytic enzymes than even large larvae, and may therefore make the food more easily ingestible or digestible to larvae.

The evolution and maintenance of parental regurgitations in burying beetles has rarely been addressed. Pukowski (1933) suggested that continued parental feeding may be necessary for the rapid development of the larvae, which she considered a prerequisite for the use of small carcasses. This argument assumes, however, that larvae cannot develop rapidly without parental care, which is not true. As Pukowski herself noted, larvae raised in the absence of an adult take no longer to leave the carcass than do larvae receiving parental care. Pukowski also argued that a brief period of parental care would have given the caring adults increased opportunities for searching for other carcasses. This hypothesis is tenable only if the evolution of parental regurgitation were preceded by the evolution of parental brood defence, because only then would accelerated larval development shorten the required period of parental defence. Systematic data unfortunately do not provide information about the sequence in which these two components of parental care evolved, since neither of them has been observed in any silphid outside the genus *Nicrophorus* (Peck 1982). If brood defence did precede regurgitation to larvae, any new trait that accelerated larval growth and consumption of the carcass would also have been favoured, because it would have reduced the time the carcass remained attractive, and concurrently, the probability of brood failure.

Regardless of the evolutionary history of these behaviours, the manufacturing of an opening in the carcass surface and regurgitation to young currently do have positive effects on breeding success. Larval mass at dispersal is a measure of offspring quality because it is highly correlated with adult body size (Bartlett & Ashworth 1988), which in turn is the prime factor affecting the outcome of competitive interactions in burying beetles (Pukowski 1933; Bartlett & Ashworth 1988; Otronen 1988; Müller et al. 1990b). Therefore, adults developing from larvae that are heavier at dispersal from the carcass are likely to be competitively superior to those developing from lighter larvae, and therefore have a better chance of securing a carcass for their own reproduction.

The benefits of parental care in *N. vespilloides* are substantial even when defensive behaviours are disregarded. The cost of parental care involves a temporal component as well as a component of risk-taking: caring beetles lose time that they could otherwise use to search for other carcasses and/or attract females for mating, and they risk severe injuries in interactions with larger congeners. The importance of the temporal investment and risk of injury depends on the future probability of finding a carcass: the lower this probability, the smaller the loss of potential future reproduction through time spent with the present brood, or through injuries that might preclude or reduce future reproductive opportunities.

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