

Carcass maintenance and biparental brood care in burying beetles: are males redundant?

JOSEF K. MÜLLER,* ANNE-KATRIN EGGERT*[†] and
SCOTT K. SAKALUK[†] *Institut für Zoologie der Albert-Ludwigs-Universität, Freiburg, Germany and

[†]Ecology Group, Department of Biological Sciences, Illinois State University, U.S.A.

Abstract. 1. Burying beetles inter small vertebrate carcasses that ultimately serve as a food source for their developing young. The male remains with the female on the carcass after the brood has been produced, purportedly to aid in the feeding and protection of larvae. However, numerous laboratory experiments have failed to demonstrate a beneficial effect of the male on the growth and survival of offspring.

2. A potential difficulty with laboratory studies is that beetles are typically held under relatively benign conditions, protected from the biotic and environmental challenges that they normally encounter. In nature, males may enhance offspring survival by aiding the female in ridding the carcass of mould, and by helping to preserve the carcass through the secretion of antibiotic substances in the beetles' saliva. To examine more rigorously the potential benefits of male parental care, an experiment was conducted under field conditions in which the reproductive output of male–female pairs was compared to that of single females.

3. Beetles were induced to bury carcasses in soil inside rigid plastic tubes that had been inserted into the ground. The experiment was a paired design involving pairs of sisters reproducing in adjacent tubes; one sister reproduced alone, whereas the other reproduced with the assistance of a male. Soil cores were recovered about 1 month later, and examined for viable pupae.

4. There was no significant difference in the number of offspring produced by single females and those reproducing with the assistance of the male, nor was there any significant difference in total brood mass. These results suggest that any benefits of extended male residency on the carcass do not stem from male participation in carcass maintenance or provisioning young.

Key words. Burying beetles, *Nicrophorus vespilloides*, parental care, reproductive success, sexual selection.

Introduction

Burying beetles (Coleoptera: Silphidae: *Nicrophorus*) inter small vertebrate carcasses that ultimately serve as a food source for the developing young (Fabre, 1899; Pukowski, 1933). Carcasses are often located by multiple individuals of both sexes, but aggressive interactions typically lead to control of the carcass by a single male–female pair (Pukowski, 1933). The resident male and female bury the carcass by passing underneath it repeatedly, creating a cavity into which the carcass eventually falls. Once underground, the carcass is

processed further by the beetles, who strip it of its fur or feathers and gradually form it into a tightly compacted carrion ball (Pukowski, 1933). Larvae hatch from eggs laid adjacent to the carcass, and make their way to the carrion ball where they are fed by the adults, who regurgitate partially-digested food directly into their mouthparts (Pukowski, 1933). The adults also excise a small opening in the integument of the carcass, allowing the larvae direct access to the interior of the carcass. As the larvae continue to feed directly on the carrion, they gradually excavate a cavity in the carcass within which they reside. Both parents are present throughout the majority of larval development, but the female remains on the carcass until larval development is complete (1–4 weeks), whereas the male typically abandons the brood a few days earlier (Bartlett, 1988; Scott & Traniello, 1990; Trumbo, 1991).

Correspondence: S. K. Sakaluk, Ecology Group, Department of Biological Sciences, Illinois State University, Normal, IL 61790–4120, U.S.A. E-mail: sksakal@rs6000.cmp.ilstu.edu

The post-copulatory behaviour of male burying beetles has long been regarded as an exemplar of paternal care, but empirical evidence to support this interpretation is equivocal (review in Eggert & Müller, 1997). In identifying potential benefits to males of their extended residency with the brood, it is useful to distinguish between fitness benefits that arise because of the improved defence of the carcass afforded by the male's presence, and those that arise through behaviours that appear to be designed to enhance the health and nutritional status of offspring, such as feeding of the young and the removal of mould from the carcass. Take-overs of buried carcasses by conspecific rivals or congeneric competitors pose a serious risk to the reproductive success of resident males, as intruders invariably kill any larvae present on the carcass upon its usurpation (Scott, 1990; Trumbo, 1990a). Field studies of *Nicrophorus defodiens* and *N. orbicollis* have shown that male–female pairs have a lower frequency of take-overs than do single females (Trumbo, 1990b, 1991; Robertson, 1993); in other studies, however, there was no difference in the retention of carcasses by pairs and single females at natural densities, and a difference emerged only when potential competitors were confined experimentally over buried carcasses (Scott, 1990, 1994).

Although the presence of a male may enhance offspring survival through improved defence of the carcass against conspecific rivals and interspecific competitors, benefits arising from the male's participation in parental care and carcass maintenance are much less clear. Several studies have failed to demonstrate a beneficial effect of the male's presence, measured in terms of the number and size of offspring produced, when potential competitors and rivals were excluded (Bartlett, 1988; Scott, 1989; Reinking & Müller, 1990; Trumbo, 1991). One criticism that can be made of all these studies, however, is that they have been conducted in the laboratory where the beetles have been held at a constant temperature in sterile peat, free of the normal environmental and biotic challenges. For example, it is known that both members of a breeding pair continually smear the carrion ball with oral and anal secretions (Pukowski, 1933) that appear to inhibit the growth of fungal hyphae and may also act as an antibacterial agent, retarding the rate of decomposition (Halffter *et al.*, 1983; Wilson & Fudge, 1984; Bartlett, 1988). If such behaviour serves to enhance the growth and survival of offspring, it is not likely to be particularly relevant in a sterile laboratory environment. Moreover, in soil where carcasses are normally buried, parents must contend with a number of other smaller arthropods that constitute potential parasites and predators of eggs and larvae (Pukowski, 1933). A fair assessment of the benefits of the male's participation in brood care and carcass maintenance requires an experiment, conducted under natural conditions, that permits the recovery of offspring of beetles allowed to reproduce in the field, and eliminates conspecific rivals and congeneric competitors as a confounding factor. The results of one such study are presented here.

Methods

The study was conducted from September to November 1994 in the Mooswald, a forest preserve near Freiburg in south-

west Germany. Burying beetles, *Nicrophorus vespilloides*, are naturally abundant at the site, but no other microphorine species were active at the time of the study. Experimental subjects were the offspring of beetles collected at the study site, and reared under standardized conditions in the laboratory (e.g. Müller, 1987; Müller & Eggert, 1990; Eggert, 1992). This protocol permitted selection of experimental subjects of known parentage, age and mating status; beetles used in experimental trials were all virgin and between 49 and 56 days old beyond the adult moult. Two experimental treatments were established, one in which a female was allowed to reproduce with the assistance of a male, and the other in which a female was given the opportunity to reproduce without the assistance of a male. The experiment was a paired design involving full sibs: for each female employed in one treatment, a full sib of that female was employed in the alternative treatment. In addition, females of each full-sib pair were inseminated by males that were themselves full sibs, but unrelated to the females. These measures were taken to control for any genetically derived variation in reproductive output, and to increase the probability of detecting any differences attributable to treatment should such exist. A total of forty-six replicates was established involving twenty-three paired females and twenty-three single females.

Experimental beetles were transported to the field site, and induced to bury 25-g mouse carcasses in soil inside rigid plastic cylinders (15 cm diameter × 20 cm long) that had been inserted into the ground prior to introducing the beetles. Cylinders were inserted into the ground with minimal disturbance to the surface litter and without disturbing the integrity of the soil beneath. A small plastic *Drosophila* tube inserted into the soil core facilitated the recovery of adults that had completed reproduction and left the carcass to search for additional reproductive opportunities; adults emerging from the soil would invariably walk around the inside wall of the cylinder, falling into the plastic tube positioned along this pathway. The cylinders were checked daily and any beetles found in the plastic vials were removed from the apparatus. Experimental adults were prevented from flying away by securing a metal strainer tightly over the top of each cylinder. The mesh size of the strainer (1 mm) precluded access by conspecific intruders and hence the possibility of take-overs. The mesh size was, however, sufficiently large to allow access of small carrion flies, which were observed on many of the carcasses prior to their burial. The forty-six cylinders were arrayed in a grid pattern with five cylinders per row; the rows and cylinders within rows were spaced 5 m apart. Full-sib females were assigned to adjacent cylinders, minimizing environmental effects across treatments.

About 20 days after experimental beetles had buried their carcasses, the soil cores were recovered from the field. Cylinders were readily extracted from the ground with their soil cores intact. These were transported back to the laboratory where the soil was slowly scraped away, thin layers at a time, to expose any beetle pupae without damaging them. The pupae were rinsed with water to remove any soil clinging to them, then weighed individually to the nearest milligram. Two correlated components of fitness, total number of offspring

produced and total brood mass, were determined for each replicate. Whenever a pupa was damaged upon its extraction from the soil, the mean mass for the remaining intact members of the brood was determined, and this value was assigned to the damaged pupa; this same protocol was adopted for larvae that had not yet pupated. Together, such cases accounted for less than 5% (34/722) of all offspring recovered.

Differences across treatments in the number of offspring produced and total brood mass were analysed using both paired *t*-tests and randomization tests; the former are more familiar to animal behaviourists, but the latter have greater statistical power (Manly, 1991). Randomization tests were conducted using RT, a statistical program for personal computers designed to perform a variety of randomization procedures (Manly, 1991, 1992). Specifically, a one-sample, paired-comparison test was used, in which the sign (negative or positive) of each observed difference between members of sibling pairs was assigned randomly, and the resulting differences summed. The randomization procedure was repeated 5000 times, as recommended by Adams & Anthony (1996). The proportion of sums generated by these multiple randomizations that was further from zero than the observed sum of differences, yielded the two-tailed probability associated with the test.

Results

Paired females produced, on average, 16.7 ± 2.3 offspring (\pm SE) ($n = 23$, range = 0–35), whereas single females produced 14.6 ± 2.7 ($n = 23$, range = 0–41). A greater proportion of single females (6/23) than paired females (2/23) failed to produce any offspring, but this difference was not significant (Fisher's exact test, $P = 0.24$). A frequency distribution of the difference in the number of offspring produced by paired females and their single siblings is shown in Fig. 1. There was no significant difference in the number of offspring produced across treatments, as assessed either by the paired *t*-test ($t = 0.71$, $P = 0.48$) or the randomization test ($P = 0.25$).

The mean total brood mass of paired females was 4.12 ± 0.61 g ($n = 23$, range = 0–9.65), compared to 3.36 ± 0.65 g for single females ($n = 23$, range = 0–8.97). A frequency distribution of the difference in total brood mass of paired females and their single siblings is shown in Fig. 2. There was no significant difference in total brood mass across treatments, as assessed by either the paired *t*-test ($t = 1.10$, $P = 0.28$) or the randomization test ($P = 0.15$).

Thomas & Juanes (1996) recommended the use of power analyses as a supplement to statistical tests that result in non-significant results. The power of the paired *t*-tests was determined at small, medium and large effect sizes, respectively, as defined operationally by Cohen (1988). Cohen (1988) provides no power tables for randomization tests, nor are such tests incorporated in the power analysis software currently available (see Thomas & Krebs, 1997). However, computer simulations have shown that randomization tests have the same or greater power than their parametric and non-parametric counterparts (Manly,

1991; Adams & Anthony, 1996), so power estimates must be regarded as conservative. Paired *t*-tests had low statistical power at small effect sizes, but moderate to high power at larger effect sizes (Table 1). Approximately 400 replicates (200 in each treatment) would be required to obtain a power of 50% at the observed effect size, which was less than the *small* effect size as defined by Cohen (1988).

Of the eight females that failed to produce any offspring, three (all single females) opted not to bury the carcass with which they had been provided. Since a female's decision to bury a carcass may be predicated, in part, on her assessment of whether a given reproductive attempt is likely to succeed, which in turn may hinge on the assistance provided by a male partner, it seemed prudent to include these cases in the comparison of the reproductive output of single and paired females. Strictly speaking, however, the study was designed to identify the benefits of a male's extended residency following carcass burial. Accordingly, the data were re-analysed, deleting values for the three sibling pairs in which the single female failed to bury the carcass. Differences between treatments remained non-significant (number of offspring: paired = 18.0 ± 2.4 vs. single = 16.8 ± 2.8 ; total brood mass (g): paired = 4.4 ± 0.6 vs. single = 3.9 ± 0.7 ; all $P = \text{NS}$ for both paired *t* and randomization tests).

Discussion

Male and female burying beetles fly independently in search of carrion and if a female locates a carcass that has not attracted any prospective mates, she may opt to bury it herself, using sperm from previous matings with which to fertilize her eggs (Pukowski, 1933; Müller & Eggert, 1989). In such instances, females tend the larvae without the assistance of a male. Experimental treatments accurately reflect therefore the uniparental and biparental breeding arrangements commonly observed in nature (e.g. Scott & Traniello, 1990; Trumbo, 1991; Eggert, 1992).

In the present study, there was no difference in the reproductive success of females reproducing with or without the assistance of a male. This result suggests that under natural conditions, a single parent is sufficient to maintain the carcass and provide the level of provisioning needed for larval development. It is possible, however, that the apparatus used to confine the beetles may have resulted in the exclusion of at least some potential competitors, thereby reducing potential benefits of a male's participation in parental care. Because each plastic cylinder formed an impenetrable sidewall around the brood chamber, competition or predation could only have come from soil arthropods initially inside the plastic wall, or those climbing under or back up through the cylinder. The strainers placed over the plastic cylinders permitted access of small carrion flies but could have been a barrier to larger species. However, large carrion flies (*Lucilia* spp., *Sarcophaga* spp.) readily oviposit or larviposit through the mesh of the strainers, leading to the production of viable larvae (personal observation), so it seems doubtful that the design of the apparatus reduced competition from flies.

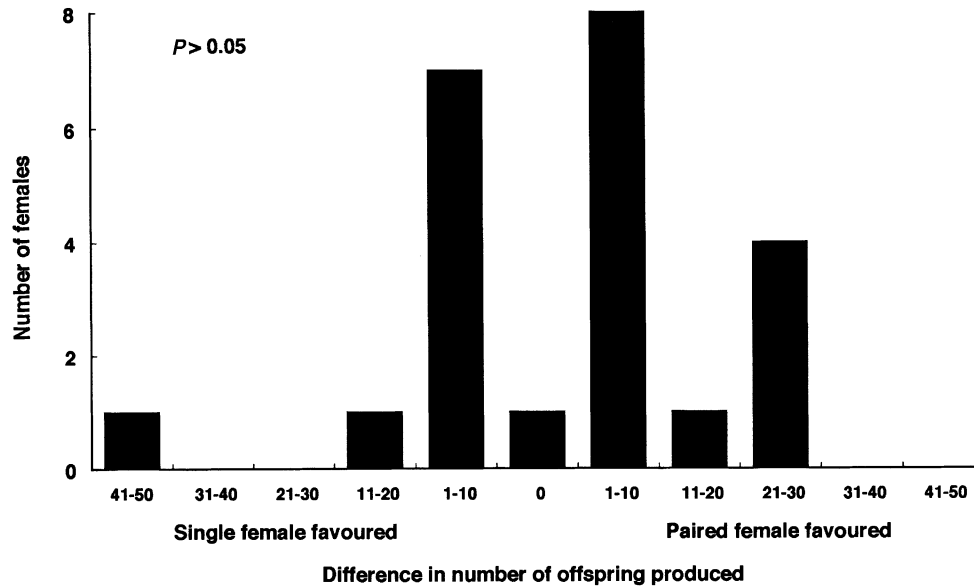


Fig. 1. Frequency distribution of the difference in the number of offspring produced by paired females and their single siblings. Bars to the right of the zero represent cases where the paired female produced more offspring; those to the left of the zero represent cases where the single female produced more offspring. There was no significant difference between the two treatments ($P = 0.25$).

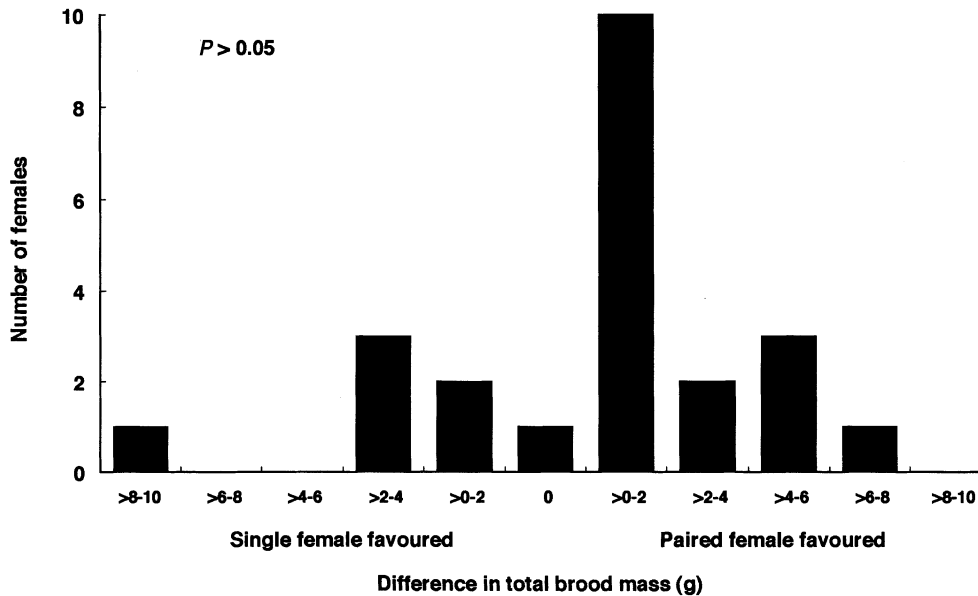


Fig. 2. Frequency distribution of the difference in total brood mass of paired females and their single siblings. Bars to the right of the zero represent cases where the total brood mass of the paired female was higher; those to the left of the zero represent cases where the total brood mass of the single female was higher. There was no significant difference between the two treatments ($P = 0.15$).

The potential benefits of parental care may vary according to spatial and temporal variation in the number of competitors. With respect to the level of competition realized in the present study, it appears that a male's presence does not appreciably enhance offspring fitness beyond that accruing through the improved defence of the carcass against conspecific rivals and congeneric competitors. However, the ubiquity of this conclusion must await the results of other field studies conducted at other times and locations.

Male burying beetles remain with females on the carcass for extended periods, even in populations where congeneric competitors are absent and the occurrence of conspecific takeovers is rare or absent (review in Eggert & Müller, 1997). Under such circumstances, the extended residency of the male appears to be paradoxical, given the absence of any benefits afforded by his presence. Given the scarcity and ephemeral nature of carrion, it could be argued that costs of an extended male residency are minimal, as a male would be unlikely to

Table 1. Power analysis of paired *t*-tests used to analyse differences in reproductive output of paired females and their single siblings. Effect sizes are as operationally defined by Cohen (1988).

Effect size	Number of offspring		Total brood mass (g)	
	Difference in number of offspring	Power	Difference in brood mass	Power
Small	2.4	0.12	0.60	0.14
Medium	6.0	0.55	1.50	0.58
Large	9.6	0.90	2.41	0.94

find another carcass in the additional time that would accrue through premature abandonment of the brood. Under such circumstances, even a slight risk of take-over may be sufficient to select against early abandonment by males. Unlike females, however, male reproduction is not wholly contingent on finding a carcass. Males can employ either of two mating tactics to attract a mate: (1) they can search for a carcass suitable for reproduction, or (2) in the absence of a carcass they can emit pheromone that attracts females solely for copulation (Müller & Eggert, 1987; Eggert & Müller, 1989a,b). Hence, males that remain with their broods on buried carcasses throughout the majority of larval development forego additional mating opportunities.

Individuals of either sex make competent parents (Bartlett, 1988; Scott, 1989; Reinking & Müller, 1990), and when providing uniparental care, males extend the duration of their residency to a period typical of females (Trumbo, 1991), and accordingly adjust the frequency at which they feed offspring (Fetherston *et al.*, 1994). It may be, therefore, that males remain with females as a form of parental insurance, providing the necessary care should the female die, become incapacitated or desert her brood prematurely (Trumbo, 1991). However, observations on the sex of parents present on carcasses exhumed with broods reveal that such contingencies are rare, if they occur at all (Scott & Traniello, 1990; Trumbo, 1991; Eggert, 1992).

Finally, it may be that the historical emphasis on the benefits of male parental care has been misplaced, as there appear to be other benefits to the extended residency of males beyond those related to care of offspring. Males may remain on carcasses throughout brood development primarily to secure additional copulations with the resident female (Eggert & Müller, 1997), or to obtain extra-pair copulations with intruding females that are subsequently expelled (Robertson, 1994). There are at least three ways that supernumerary copulations with the resident female might benefit a male remaining on a carcass. First, if a female is required to produce a replacement brood upon the failure of the first to hatch (Müller, 1987), a male increases the probability that his sperm will have precedence in subsequent fertilizations (Bartlett, 1988; Müller & Eggert, 1989; Scott & Williams, 1993). Second, repeated copulations may reduce sperm competition from satellite males, defeated rivals that remain in the vicinity of the carcass and attempt surreptitious copulations with the female (Dressel, 1987; Bartlett, 1988). Finally, copulations that occur even after the

majority of brood development has been completed may increase a male's fertilization success in subsequent batches of eggs laid by females upon the location of other carcasses. This would be especially important in those cases in which the female locates a carcass on which no male is present, because females readily bury carcasses in the absence of a mate, using stored sperm from previous matings to fertilize their eggs (Pukowski, 1933; Müller & Eggert, 1989). In conclusion, until the potential benefits to males of multiple matings have been assessed, efforts to assign a parental care function to male postcopulatory behaviour should be regarded as premature.

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References

- Adams, D.C. & Anthony, C.D. (1996) Using randomization techniques to analyse behavioural data. *Animal Behaviour*, **51**, 733–738.
- Bartlett, J. (1988) Male mating success and paternal care in *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Behavioral Ecology and Sociobiology*, **23**, 297–303.
- Cohen, J. (1988) *Statistical Power Analysis for the Behavioral Sciences*, 2nd edn. Lawrence Erlbaum Associates, Hillsdale, New Jersey.
- Dressel, J. (1987) The influence of body size and presence of females on intraspecific contests of males in the carrion beetle *Nicrophorus vespilloides* (Coleoptera, Silphidae). *Verhandlungen der Deutschen Zoologischen Gesellschaft*, **80**, 307.
- Eggert, A.-K. (1992) Alternative male mate-finding tactics in burying beetles. *Behavioral Ecology*, **3**, 243–254.
- Eggert, A.-K. & Müller, J.K. (1989a) Pheromone-mediated attraction in burying beetles. *Ecological Entomology*, **14**, 235–237.
- Eggert, A.-K. & Müller, J.K. (1989b) Mating success of pheromone-emitting *Nicrophorus* males: do attracted females discriminate against resource owners? *Behaviour*, **110**, 248–257.
- Eggert, A.-K. & Müller, J.K. (1997) Biparental care and social evolution in burying beetles: lessons from the larder. *The Evolution of Social Behaviour in Insects and Arachnids* (ed. by J. C. Choe and B. J. Crespi), pp. 216–236. Cambridge University Press, Cambridge.
- Fabre, J.-H. (1899) *Souvenirs Entomologiques*. Librairie Delagrave, Paris.
- Fetherston, I.A., Scott, M.P. & Traniello, J.F.A. (1994) Behavioural compensation for mate loss in the burying beetle *Nicrophorus orbicollis*. *Animal Behaviour*, **47**, 777–785.
- Halfiter, G., Anduaga, S. & Huerta, C. (1983) Nidification des *Nicrophorus*. *Bulletin de la Société Entomologique de France*, **88**, 648–666.
- Manly, B.F.J. (1991) *Randomization and Monte Carlo Methods in Biology*. Chapman & Hall, London.

- Manly, B.F.J. (1992) *RT. A Program for Randomization Testing, Version 1.02*. West, Inc., Cheyenne, Wyoming.
- Müller, J.K. (1987) Replacement of a lost clutch: a strategy for optimal resource utilization in *Necrophorus vespilloides* (Coleoptera: Silphidae). *Ethology*, **76**, 74–80.
- Müller, J.K. & Eggert, A.-K. (1987) Effects of carrion-independent pheromone emission by male burying beetles (Silphidae, *Necrophorus*). *Ethology*, **76**, 297–304.
- Müller, J.K. & Eggert, A.-K. (1989) Paternity assurance by 'helpful' males: adaptations to sperm competition in burying beetles. *Behavioral Ecology and Sociobiology*, **24**, 245–249.
- Müller, J.K. & Eggert, A.-K. (1990) Time-dependent shifts between infanticidal and parental behavior in female burying beetles: a mechanism of indirect mother–offspring recognition. *Behavioral Ecology and Sociobiology*, **27**, 11–16.
- Pukowski, E. (1933) Ökologische Untersuchungen an *Necrophorus* F. *Zeitschrift für Morphologie und Ökologie der Tiere*, **27**, 518–586.
- Reinking, M. & Müller, J.K. (1990) The benefit of parental care in the burying beetle, *Necrophorus vespilloides*. *Verhandlungen der Deutschen Zoologischen Gesellschaft*, **83**, 655–656.
- Robertson, I.C. (1993) Nest intrusions, infanticide and parental care in the burying beetle, *Necrophorus orbicollis* (Coleoptera: Silphidae). *Journal of Zoology (London)*, **231**, 583–593.
- Robertson, I.C. (1994) Extra-pair copulations in burying beetles (Coleoptera: Silphidae). *Journal of the Kansas Entomological Society*, **67**, 418–420.
- Scott, M.P. (1989) Male parental care and reproductive success in the burying beetle, *Necrophorus orbicollis*. *Journal of Insect Behavior*, **2**, 133–137.
- Scott, M.P. (1990) Brood guarding and the evolution of male parental care in burying beetles. *Behavioral Ecology and Sociobiology*, **26**, 31–39.
- Scott, M.P. (1994) Competition with flies promotes communal breeding in the burying beetle, *Necrophorus tomentosus*. *Behavioral Ecology and Sociobiology*, **34**, 367–373.
- Scott, M.P. & Traniello, J.F.A. (1990) Behavioural and ecological correlates of male and female parental care and reproductive success in burying beetles (*Necrophorus* spp.). *Animal Behaviour*, **39**, 274–283.
- Scott, M.P. & Williams, S.M. (1993) Comparative reproductive success of communally breeding burying beetles as assessed by PCR with randomly amplified polymorphic DNA. *Proceedings of the National Academy of Sciences (USA)*, **90**, 2242–2245.
- Thomas, L. & Juanes, F. (1996) The importance of statistical power analysis: an example from *Animal Behaviour*. *Animal Behaviour*, **52**, 856–859.
- Thomas, L. & Krebs, C.J. (1997) A review of statistical power analysis software. *Bulletin of the Ecological Society of America*, **78**, 126–139.
- Trumbo, S.T. (1990a) Reproductive benefits of infanticide in a biparental burying beetle *Necrophorus orbicollis*. *Behavioral Ecology and Sociobiology*, **27**, 269–273.
- Trumbo, S.T. (1990b) Interference competition among burying beetles (Silphidae, *Necrophorus*). *Ecological Entomology*, **15**, 347–355.
- Trumbo, S.T. (1991) Reproductive benefits and the duration of paternal care in a biparental burying beetle, *Necrophorus orbicollis*. *Behaviour*, **117**, 82–105.
- Wilson, D.S. & Fudge, J. (1984) Burying beetles: intraspecific interactions and reproductive success in the field. *Ecological Entomology*, **9**, 195–203.

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