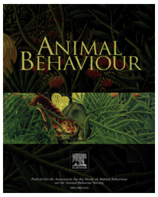
Animal Behaviour 118 (2016) 47–54

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav



Parental care and competition with microbes in carrion beetles: a study of ecological adaptation

Stephen T. Trumbo [a], [*], Derek S. Sikes [b], Paula K. B. Philbrick [a]

[a] Department of Ecology and Evolutionary Biology, University of Connecticut, Waterbury, CT, U.S.A.
[b] University of Alaska Museum, University of Alaska Fairbanks, AK, U.S.A.

ARTICLE INFO

Article history:
Received 1 December 2015
Initial acceptance 20 January 2016
Final acceptance 21 April 2016
Available online 22 June 2016
MS. number: A15-01015R

Keywords:
adaptation
animal–microbe interaction
carrion ecology
comparative method
microbial ecology
Nicrophorus
parental care
Silphidae

The investigation of the function of a complex adaptation can benefit from multiple approaches. We used comparative study and physical simulation to re-examine the hypothesis that parental adaptations enable carrion beetles (Silphidae) to better compete with carrion microbes. The comparative study used two closely related species, *Nicrophorus orbicollis* and *Ptomascopus morio*, which differ markedly in their level of care. Reproductive performance was measured using carcasses aged on field soil for 0, 3, 6 or 9 days. As expected, *N. orbicollis* converted more carrion biomass to offspring biomass than did *P. morio* (27.8% versus 16.6% on fresh carcasses) and both species produced smaller broods on aged carcasses. There was a negative interaction, however, between the level of care and carcass age on reproductive performance; the parental care advantage exhibited by *N. orbicollis* was less on older carcasses than on fresh carcasses, and was negligible on the oldest (6- and 9-day) carcasses. *Ptomascopus morio* also demonstrated superior ability to regulate the size of offspring when using carcasses of various ages. In a second experiment, we simulated antimicrobial activity of *Nicrophorus* anal exudates. A 6-day pretreatment with an antibacterial, an antifungal, or a combination treatment did not result in larger broods relative to those on untreated 6-day carcasses. The results call into question the hypothesis that preparation of the carcass by *Nicrophorus* evolved primarily as an adaptation for preventing microbial deterioration of carrion. We suggest that selection to compete with microbes may be relaxed in carrion beetles that provide elaborate parental care. A small vertebrate carcass is a potential model system for applying the comparative method to ecological function.

Complex biological adaptations have multiple potential functions. The investigation of function presents a challenge because adaptations are entwined in a web of ecological correlates, and these change over evolutionary time. Parental care serves many roles in vertebrates (Balshine, 2012). Although invertebrate parental care may appear less developed and can be argued to have a single prime mover in a particular system (Wilson, 1975), once care originates, additional functions evolve (Trumbo, 2012). The study of the utility of parental adaptations is further complicated because during the evolution of parental care, care may replace functions that were previously met by traits less explicitly parental. For example, the utility of parental care may be easily demonstrated in some cases by removing the parent, resulting in near 100% offspring mortality from predators (Tallamy & Denno, 1981), parasitoids (Edgerly, 1997) or microbes (Kudo, Akagi, Hiraoka, Tanabe, & Morimoto, 2011). Ancestors that did not provide such care obviously had alternative mechanisms to protect their young (Zeh, Zeh, & Smith, 1989). A complete study of a parental or other social adaptation would include not only tests of alternative hypotheses of current utility but would examine whether the trait is novel (apomorphic, Coddington, 1988) in care-providing lineages. Although rarely done, it would also assess whether the novel trait has lesser, equal or greater function compared to related species without explicit care.

The present study takes two approaches, simulation and comparative, to examine a previously investigated complex parental trait, the postburial preparation of a small vertebrate carcass by burying beetles (*Nicrophorus* spp.). A physical simulation can isolate one component of a complex behaviour to evaluate utility. Zoran and Ward (1983) employed artificial agitation of water to relate fanning behaviour in a care-giving fish to increased oxygen consumption and faster development of embryos. Comparative studies can be either broad phylogenetic contrasts

---

[*] Correspondence: S. T. Trumbo, Department of EEB, University of Connecticut, Waterbury, CT 06702, U.S.A.
E-mail address: trumbo@uconn.edu (S. T. Trumbo).

http://dx.doi.org/10.1016/j.anbehav.2016.06.001
0003-3472/

(Losos, 2011) or a narrow contrast of related species differing in a key trait. A classic example of a narrow contrast is the evolutionary divergence in mobbing behaviour of predators by gulls, intense in open-nesting species and nearly absent in cliff-nesting kittiwakes that have little to fear from predators (Cullen, 1957). A narrow contrast, as in the present study, can also be a side-by-side experiment under controlled conditions. Birds that frequently cache seeds demonstrate superior spatial memory to species that rarely cache seeds (Balda & Kamil, 1989). Comparative analysis is employed more frequently to evaluate behavioural, morphological or physiological adaptations rather than ecological adaptations such as the ability to compete. The shared ecosystem of a small carcass, however, is sufficiently contained to allow experimental comparison of the ability of species with and without developed parental care to cope with an equivalent challenge from the microbial community.

The preparation of a small vertebrate carcass by burying beetles for their offspring includes application of anal and oral secretions. Pukowski (1933) suggested three functions for anal exudates: retaining moisture, aggregating larvae on the carcass and deterring carrion competitors by advertising the presence of burying beetles. Three more recent hypotheses are that anal exudates may reduce odour cues from the carcass (Suzuki, 1999), provide nutritive enzymes for the young (Degenkolb, Düring, & Vilcinskas, 2011) or are part of a social immune response to protect against pathogens (Cotter, Littlefair, Grantham, & Kilner, 2013). The hypothesis that has received the most study and support, however, is that anal exudates are a counteradaptation to microbes of decay, preserving the resource for burying beetle young (Arce, Johnston, Smiseth, & Rozen, 2012; Rozen, Engelmoer, & Smiseth, 2008; Scott, 1998). The reasoning follows Janzen's (1977) hypothesis that microbes compete with animals by making carrion unusable, both by direct consumption and by production of toxins.

The anal exudates of *Nicrophorus* contain lysozymes that inhibit growth of Gram-positive bacteria (Arce et al., 2012), an anti-Gram negative component (Hall et al., 2011), and an antifungal agent (Hwang & Lin, 2013; see; Suzuki, 2001). Each of these studies frames anal exudates as an adaptation that helps burying beetles win more of the resource from microbes by killing or slowing growth of microbes and combatting toxic effects. A nongonadal gland that waxes and wanes during the parental cycle is a possible source of components of the exudate (Huerta, 1991). While demonstrations of antimicrobial activity against individual species of microbes are of interest, it is difficult to predict the community-level response because of complex positive and negative interactions among microbes (O'Malley, 2014). Extrapolation to the community might be especially difficult in burying beetles because their anal exudates, in addition to containing antimicrobials, include a rich assemblage of microbes (Kaltenpoth & Steiger, 2014). A prepared carcass hosts a qualitatively different, and not just a diminished, microbial community compared to an untreated carcass. To date, there have been few tests of alternative hypotheses for function, little simulation of the effect of antimicrobial applications to carrion (but see Arce et al., 2012) and no comparative study of close relatives that do not prepare the carcass for their young.

Here, we attempt to broaden the approaches to the study of antimicrobial traits in burying beetles, a perspective that can enhance understanding of both current function and evolutionary history (Forber & Griffith, 2011; Olson & Arroyo-Santos, 2015). We compare the reproductive performance of two species in closely related genera, one that removes hair, rounds the carcass and applies anal and oral exudates (*Nicrophorus orbicollis* Say) and one that does not (*Ptomascopus morio* Kraatz). We estimate the two expected outcomes that parental care in *Nicrophorus* enhances the conversion of carrion biomass to offspring biomass, and that aged carcasses result in lower breeding success in both species. The more interesting result is the interaction between species (level of care) and carcass age on reproductive performance. A positive statistical interaction between the level of care and carcass age would be consistent with parental care being an effective adaptation for utilizing an older, microbe-rich carcass. No statistical interaction would suggest that care has little impact, and a negative interaction would be evidence that a care-giving silphid species has less ability to compete with microbes on older carcasses than a silphid without elaborate care. We also used antibacterial and antifungal preparations to simulate the effect of application of anal exudates during the prenatal period by parent burying beetles. The results suggest that a high level of antimicrobial function does not depend on parental care in this group and that competition with microbes may not have been the primary selective force promoting the evolution of carcass preparation behaviour (cf. Arce et al., 2012; Rozen et al., 2008).

METHODS

Evolutionary and Natural History of Carrion Beetles (Silphidae)

Most species of beetles in the family Silphidae feed on carrion and/or carrion-feeding insect larvae, particularly calliphorid fly larvae (Ratcliffe, 1996). Species of the subfamily Silphinae, none of which have developed parental care, typically breed on large carcasses, past the fresh stage of decomposition, where they and their larvae feed on fly maggots and carrion. Their larvae are well sclerotized and mobile. The subfamily Nicrophorinae is the sister lineage to the Silphinae and includes species that specialize on small fresh carcasses. The vast majority of the Nicrophorinae are in the genus *Nicrophorus*, exhibiting highly developed parental care. The genus *Ptomascopus*, also a nicrophorine, has a number of intermediate characteristics including moderately sclerotized larvae, use of small to mid-sized carcasses for breeding and traits inferred as homologous with those of *Nicrophorus*: stridulation, release of sex pheromone and shorter elytra than silphines (Sikes, 2003, 2005). Sikes and Venables (2013), using fossil calibration dates from Cai et al. (2014), inferred the phylogeny of the subfamily and estimated that the family Silphidae dates to 186–164 Ma and the genus *Nicrophorus* (and presumably the origin of complex care) dates to 127–99 Ma (mid- to lower Cretaceous).

When a female burying beetle discovers a carcass, she initially refrains from feeding while she prepares the carcass by burying, rounding and removing hair. She then applies anal and oral secretions to the surface of the carcass. Only at this time does she take her first meal, and upon finishing, immediately repairs the feeding hole and reapplies secretions (Pukowski, 1933). The female then lays her eggs in the nearby soil, and upon hatching, the larvae must locate the carcass. Shortly before the larvae begin to hatch, a hole is opened through the carcass skin to allow the larvae access to the resource. Larvae receive regurgitations from parents but they also self-feed from the prepared resource (Smiseth, Darwell, & Moore, 2003). The genus *Ptomascopus* is the sister lineage to *Nicrophorus* + *Eonecrophorus* Kurosawa (1985). Only one of the three species of *Ptomascopus* has been well studied, viz. *P. morio*. This species acts as a brood parasite of *N. concolor* Kraatz and can breed on its own on either small or medium-sized carcasses (Nagano & Suzuki, 2008; Trumbo, Kon, & Sikes, 2001). In common with *Nicrophorus*, *P. morio* has adaptations for utilizing small carcasses, including release of sex pheromone, the ability to adjust clutch size to the size of the carcass and guarding a carcass by attacking potential predators (Suzuki & Nagano, 2006). *Ptomascopus morio* does not bury or round a carcass, remove hair or provide care in the form of anal