

ORIGINAL ARTICLE

Males are worse mothers: Comparing care patterns in a facultatively caring beetle

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Abstract During biparental pre- and post-hatching care, parents take on energy-consuming tasks for the offspring's benefit and further reduce their own individual costs by specializing in different care aspects. But how is biparental care evolutionarily stable when biparental care is facultative, that is, when offspring survival does not obligately rely on post-hatching care? We examine this phenomenon in the carrion-breeding beetle *Nicrophorus vespilloides*, whose facultative biparental care involves microbiome control of the carcass nursery through continued application of antimicrobial exudates, shielding offspring from adverse environmental conditions. While evidence suggests synergistic effects of biparental care in *Nicrophorus*, any adaptive benefits in terms of social immunity are unknown in this genus. We presented *Nicrophorus* adults with a microbial challenge while manipulating parental care patterns during the period of post-hatching care, investigating consequences in parent and offspring performance. We found that microbial environment and parental care pattern influence larval development and survival. Additionally, we show for the first time that both factors affect personal immunity response in *Nicrophorus* offspring, responding to challenging conditions. Simultaneously, we show that biparentally caring beetles lose more weight during post-hatching care than uniparentally caring beetles, indicating higher investment and/or higher competition with mates or offspring. We present new evidence that burying beetle offspring adjust their personal immunity based on their microbial and social environment, and that biparental care may allow parents to sustain parental care under challenging conditions, raising further questions about the interplay of care patterns and the microbial environment on immune-regulatory and developmental processes in offspring.

Key words biparental care; evolutionary biology; insect immunity; *Nicrophorus*; offspring immunity; social immunity

Introduction

Parental care, defined by Smiseth *et al.* (2012) as any parental trait that enhances offspring fitness such as food provisioning, nest construction, or defense against predators or pathogens, comes with fitness costs to caring parents. In some species, parents divide their care efforts during biparental care, which reduces the fitness costs in-

curred on each parent (Trivers, 1972). Biparental care occurs across several taxa, for example, mammals, reptiles, amphibians and insects (Ginsberg & Huck, 1989; Adams *et al.*, 2005; Uller & Olsson, 2008; Potticary *et al.*, 2024), but is still relatively rare despite the apparent benefit of a reduced energy investment, apart from birds, where it is exhibited in over 90% of species (Cockburn, 2006). This scarcity is likely due to the nature of sexual conflict: during biparental care, parents benefit from the investment of their partner and their own, while only paying for their own effort, a situation which can facilitate and reward exploitation of the partners' efforts (Trivers, 1972;

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Parker, 2006; Harrison *et al.*, 2009). To explain the occurrence of stable biparental care, different conflict resolution strategies have been proposed such as the early “sealed bid models” (Houston & Davies, 1985) where each parent’s efforts are fixed beforehand, or the later, more comprehensive “negotiation models” which postulate that each parent matches or compensates for the investment of their partner (McNamara *et al.*, 2003; Johnstone & Hinde, 2006). Overall, however, males generally invest less into care throughout the animal kingdom (except in fishes), in part due to uncertainty of their own paternity and higher opportunity costs (Kokko & Jennions, 2012; Søraker *et al.*, 2023) resulting from multiple matings by females in species that exhibit parental care (Adams *et al.*, 2005; Uller & Olsson, 2008).

To be an evolutionarily stable strategy, facultative biparental care must outweigh the costs of forgone breeding opportunities for parents (Maynard Smith, 1977; Székely & Cuthill, 1999) or at least double offspring fitness (Maynard Smith, 1977; Grafen, 1990; Yamamura & Tsuji, 1993), which can occur, for example, in face of high mortality or severe competition. For instance, males of *Nicrophorus* burying beetles increase their future reproductive chances by remaining with their brood longer by gaining access to the shared resources (Chemnitz *et al.*, 2017) but can also increase offspring fitness by staying with the brood to guard their investment in face of high intraspecific competition (Trumbo, 2007). Importantly, parental care is selected for to primarily benefit offspring, for instance by increasing immunocompetence, for example in mice and rats (Michaut *et al.*, 1981; von Hoersten *et al.*, 1993). It also enhances survival, demonstrated across many different species including insects, such as earwigs (Kölliker, 2007) and burying beetles (Grew *et al.*, 2019). Intriguingly, the question of whether offspring benefit from biparental care more than uniparental care is still being debated (Houston & Davies, 1985; McNamara *et al.*, 2003; Barta *et al.*, 2014). Theory suggests that under the “negotiation” model of biparental care coordination, biparental care may reduce (Lessells & McNamara, 2011) or increase (Johnstone *et al.*, 2014) offspring fitness. Recent evidence in *N. vespilloides* suggests that biparental care may provide synergistic benefits to offspring through higher offspring growth and survival (Pylakouta *et al.*, 2018). Similarly, evidence in *N. orbicollis*, which obligately relies on post-hatching care, shows that biparental pairs are associated with larger broods when allowed to freely choose their care patterns (Benowitz & Moore, 2016). However, whether other mechanisms of post-hatching care in addition to brood guarding and more frequent feeding are driving any such effect, such

as microbial management by parents, remains unknown. Furthermore, the question of individual costs incurred by biparental care (Müller *et al.*, 2007; Creighton *et al.*, 2009), as well as additional benefits beyond those derived through task compensation and task specialization (Scott, 1990; Creighton *et al.*, 2015), remains largely open.

Nicrophorus burying beetles are uniquely suited to investigate the benefits of different care patterns as uniparental and biparental care co-occur in the same population (Eggert, 1992). *Nicrophorus* engages in elaborate pre-hatching (burial, preparation and defense of the carcass resource) and post-hatching care (food provisioning of larvae, carcass maintenance and brood guarding) with specialized roles for each sex (Walling *et al.*, 2008; Ratz, 2025). Importantly, all care tasks can be performed by either parent, but uniparental care of either sex is less common, especially for males, where studies report it in only 5% of cases (Eggert, 1992; Smiseth & Moore, 2004). Furthermore, larvae of some burying beetle species, such as *N. vespilloides*, do not obligately rely on post-hatching care and exhibit survival rates of 50% without it (Capodeanu-Nägler *et al.*, 2016). During care, parents and offspring are exposed to the carcass microbiome and associated products of decomposition (Arce *et al.*, 2013; Jacobs *et al.*, 2014; Körner *et al.*, 2023), an environment in which parental care provides key mechanisms alleviating the microbial load of the nursery. Larvae and parents both contribute to social immunity in an effort to protect the carcass nursery from microbial decay until offspring start to disperse and pupate (~7–10 d after hatching) (Hoback *et al.*, 2004; Degenkolb *et al.*, 2011; Körner *et al.*, 2023; Müller *et al.*, 2025). Social immunity is defined as “any collective or personal mechanism that has emerged and/or is maintained at least partly due to the antipathogen defense it provides to homospecific group members” (Van Meyel *et al.*, 2018) and may be a necessary component in the evolution of sociality, ranging from more complex forms of society to earlier, simpler forms, such as facultative family life (Cremer *et al.*, 2007; Cotter & Kilner, 2010a; Meunier, 2015; Cremer *et al.*, 2018; He *et al.*, 2018; Jones *et al.*, 2018). However, how biparental care shapes personal and social immunity in caring parents and their offspring remains poorly understood.

Parental care and immunity are closely linked in *Nicrophorus*, as parental deprivation reduces lytic activity of larval exudates and influences their gene expression profile (Reavey *et al.*, 2014; Ziadie *et al.*, 2019), but parental care is not split evenly as females provide more direct care to larvae while males focus on carcass and brood defense (Scott, 1990; Trumbo, 2007;

Potticary *et al.*, 2024). Males greatly increase direct care upon mate loss, while females do not, potentially as they are already at maximum capacity (Creighton *et al.*, 2015). Uniparental and biparental care have been considered equal as larvae typically do not differ in weight at the time of carcass dispersal (Smiseth *et al.*, 2005). A recent study demonstrated synergistic effects of joint care, showing that larvae benefit from the presence of both parents when per-parent workload is experimentally kept constant (Pilakouta *et al.*, 2018). However, synergies or benefits of biparental care under more natural conditions—where workload in terms of carcass and brood size is determined before any post-hatching care pattern emerges—has not yet been demonstrated. To our knowledge, no study has investigated these effects in terms of social and offspring immunity despite their likely importance in family life in this genus (Ziadie *et al.*, 2019; Körner *et al.*, 2023).

To investigate how biparental care differs to uniparental care and how it shapes parental, social, and offspring immunity, we provided *N. vespilloides* breeding pairs with either a fresh (1 d old) or an aged (7 d old) carcass, while simultaneously manipulating the post-hatching care pattern (female, male or both parents). We measured performance and immunity of larvae and adults 24 h after larvae arrived at the carcass. The microbial challenge of an aged carcass may force adults to increase their contribution to social immunity while it should negatively influence larval performance (Rozen *et al.*, 2008; Körner *et al.*, 2023). In this case, we expect a higher energy investment of parents in the uniparental care treatment, as they have to compensate for a missing partner. Additionally, larvae under uniparental male care should fare equally well to larvae receiving uniparental female or biparental care. We also expect aged carcasses to result in parents and offspring increasing their investment into personal immunity.

Materials and methods

Beetle husbandry

N. vespilloides beetles in this study were 10th generation descendants of beetles collected near Bayreuth, Germany (49°55'10.8"N, 11°34'22.0"E). Beetles were kept under standard laboratory conditions in a climate chamber (20 °C, 16 h L : 8 h D rhythm) with up to five same sex siblings in one box (10 cm × 10 cm × 6 cm). They were fed twice a week with thawed fly larvae (*Lucilla cuprina*), with three to four larvae per beetle. To prevent inbreeding, the population was regularly infused with

fresh-caught beetles and breeding pairs were chosen for optimal outbreeding using Kinshipper (www.kinshipper.com, Bayreuth).

Experimental design

To investigate if the parental care pattern and/or carcass age influence the immunity of *N. vespilloides* offspring and parents, we employed a 2 × 3 full-factorial design (fresh or aged carcass × uniparental male care, uniparental female care or biparental care) with a total of 120 breeding beetle pairs (± 25 d old). For the aged carcass treatment, frozen mice were put into plastic boxes (10 cm × 10 cm × 6 cm) on top of coconut peat (similar to Rozen *et al.*, 2008; Jacobs *et al.*, 2014). They were then placed in a dark climate cabinet (20 °C) for 7 d before transferring them to new boxes containing fresh coconut peat. Due to their continuous decay and higher microbial load, aged carcasses represent a challenge for the beetles, requiring an increased investment into their social immunity capabilities (Janzen, 1977; Rozen *et al.*, 2008; Cotter *et al.*, 2010; Jacobs *et al.*, 2014). For the fresh carcass treatment, mice were thawed overnight at 4 °C prior to treatment start and then put into plastic boxes with fresh coconut peat. Using a caliper (accuracy ± 0.03 mm), the pronotum of each individual beetle was measured as an approximation of body size, afterward, beetle pairs were set up with a carcass (aged or fresh) and remained under light for 1.5 h (which induces the burial behavior) before being transferred to a dark climate cabinet (20 °C). Here beetles started to breed and both parents conducted pre-hatching care in form of carcass preparation (Potticary *et al.*, 2024). All mice carcasses (12 ± 1.5 g, frostfuter.de) were stored at −20 °C prior to the experiment.

Oviposition and embryonic development occur over 3 d in *N. vespilloides*, during which parents continue to prepare and maintain the carcass resource. Two days after setting up beetle pairs (after oviposition but before larval hatching), we transferred parents and carcass to a new box with fresh coconut peat, separating them from the eggs. After 24 h we checked each box with eggs every 2 h for hatched larvae on the top layer of the coconut peat. These larvae were collected on moist filter paper in a petri dish at 4 °C to slow their metabolism and development. Pooling offspring like this allows controlling arrival and size of the brood and excludes any genetic effects and age differences between larvae (Prang *et al.*, 2022). Larvae were pooled separately for each carcass treatment to maintain any maternal effects on the eggs based on the carcass age, as “priming” of offspring through maternal effects may obfuscate any effects the immediate environ-

ment has on offspring performance if offspring are suddenly subjected to different conditions.

We then kept either both parents (biparental care, $n = 38$), removed the female (uniparental male care, $n = 35$) or male beetle (uniparental female care, $n = 36$) from the carcass just before adding a brood of 15 larvae (of the corresponding carcass age treatment) to each carcass. Removing a parent as larvae are given to the carcass standardizes the pre-hatching care between treatments and mimics desertion or mortality during post-hatching care. In *N. vespilloides*, brood size is determined by carcass size during pre-hatching care, up to quasi-maximum of 30 larvae on 10 g carcass (Müller *et al.*, 1990). The carcass size in our study was chosen to allow a brood of 15 larvae to survive until dispersal under normal circumstances (biparental care and fresh carcass) (Bartlett & Ashworth, 1988) while limiting resources to a point where trade-offs are observable but culling of the brood is prevented. In contrast to a previous study (Pilakouta *et al.*, 2018), we chose not to keep workload per parent constant. This is because in the field, carcass size and brood size are determined before and during pre-hatch care and do not depend on or result in specific post-hatching care patterns (uni- or biparental care). Here we aim to measure the effects of workload variation that comes with different care patterns on parents and offspring. Prior to the addition of larvae, fostering parents and larvae were weighed. We only added larvae to parents whose own eggs had begun hatching to prevent infanticide (Eggert & Müller, 2011), as *Nicrophorus* beetles do not distinguish between foster and genetically related offspring if larvae arrive at the carcass during the correct time (Müller & Eggert, 1990). Beetles and larvae were then returned to the climate cabinet (20 °C and darkness). After 24 h, we weighed and counted surviving larvae, as well as weighing the foster parent(s), before sampling larvae and parents for our immune assays.

Whole-body larval sampling

To extract larval hemolymph for phenoloxidase (PO) and lytic activity assays, we randomly selected 5 larvae out of the whole brood and transferred them into a 1.5 mL Eppendorf reaction tube. The tube was then submerged in liquid nitrogen, freeze-killing the larvae instantly. Reaction tubes were recovered and 100 μ L of 5 mmol/L MOPS buffer with protease inhibitor (cOmpleteTM protease inhibitor, Roche) was added. Larvae were homogenized using a plastic pistil and the homogenate was put on ice before storage at -80 °C until later use.

Larval hemolymph extraction and hemocyte counting

To extract larval hemolymph for hemocyte counting, larvae were placed in a petri dish beneath a stereo microscope. Using a glass needle, we breached the soft cuticle of the larvae while holding them in place with soft-steel tweezers, applying light pressure to the larvae's body. Emerging hemolymph formed a droplet and was quickly collected using a 5 μ L glass capillary (Hirschmann ringcaps[®]). Hemolymph of three different larvae of the same brood was pooled and diluted 1 : 7.5 with Mas-EDTA (9 mmol/L EDTA, 27 mmol/L sodium citrate, 336 mmol/L NaCl, 115 mmol/L glucose, pH = 7). Next, we added 2.35 μ L of trypan blue (Sigma-Aldrich) per 10 μ L of hemolymph sample to differentiate between living and dead cells. Hemocytes were counted using a disposable hemocytometer (Neubauer improved, C-Chip, 4 chambers, NanoEntek) and a microscope (Stemi 508, Carl Zeiss Microscopy GmbH).

Parental hemolymph and exudate extraction

Oral exudates are often readily produced by burying beetles as a defense mechanism when simply handling the beetles and can then easily be collected using 5 μ L glass capillaries. Alternatively, beetles can be enticed to release exudates by slightly disturbing their antennae with air (i.e., gently blowing air on their head). Collected exudates were diluted 1 : 10 with 5 mmol/L MOPS buffer (protease inhibitor added) and placed on ice. To extract hemolymph for lytic and PO assays, we pierced the soft cuticle on the ventral side of the mesothorax with a needle cleaned with ethanol (70%). Exiting hemolymph formed a droplet on the cuticle and was collected with a 5 μ L glass capillary (Hirschmann ringcaps[®]). Hemolymph was diluted 1 : 10 with 5 mmol/L MOPS buffer (protease inhibitor added) and placed on ice. Hemolymph and exudate samples were stored at -80 °C until further use.

PO activity assays

To measure the activity of PO in the hemolymph of adults and larvae homogenate, 10 μ L of sample, 10 μ L of double-distilled water and 10 μ L of 5 mmol/L MOPS buffer (no protease inhibitor added) were added to each well of 96-well plate while on ice. As a next step, we added 180 μ L L-DOPA (L-3,4-dihydroxyphenylalanine) solution in two steps, mixing everything by carefully pipetting up and down. L-DOPA solution was freshly prepared beforehand by dissolving 39.4 mg of L-DOPA in 20 mL of 5 mmol/L MOPS buffer (no protease inhibitor

added). The solution was vortexed for 20 min and put in an ultrasonic bath for 3 min, as L-DOPA is difficult to dissolve, before sterile filtering it (syringe filter, 0.45 μ m, VWR). The PO activity of the samples was then measured at 490 nm over a period of 2 h every 2 min using a microplate reader (Synergy H1 Microplatereader, Biotek). We excluded samples with an R^2 -value (goodness of fit) of less than 0.8, and samples with discontinuous activity curves.

To measure total-PO activity, prophenoloxidase contained in larval homogenate was chemically activated by chymotrypsin (Cerenius & Söderhäll, 2004). Preparation was done as outlined above but MOPS-buffer was substituted with 10 μ L of chymotrypsin solution (6.67 U/mL in 5 mmol/L MOPS buffer without protease inhibitor). Plates were incubated at room temperature for 5 min to facilitate cleavage into active PO. Afterward, 180 μ L of L-DOPA solution was added as described above. Total-PO activity was then measured identically to activity measurements of free PO.

Lytic activity assays

Lytic activity was measured by adding 10 μ L exudate sample to the wells of 96-well plate and adding 90 μ L of *Micrococcus luteus* solution (3 mg of freeze-dried *M. luteus* in 10 mL of MOPS buffer). The OD₆₀₀ was measured over a period of 2 h at 2 min intervals using a microplate reader (Synergy H1 Microplatereader, Biotek). During our analysis, we excluded samples with an R^2 value (goodness of fit) of less than 0.8, and samples with discontinuous activity curves.

Statistical analysis

Data analysis was conducted in RStudio version 2024.12.0.467 (Posit team, 2024) using R version 4.3.3 (R Core Team, 2022), loaded with packages DHARMA 0.4.6 (Hartig, 2022), lme4 1.1-35.3 (Bates *et al.*, 2015), emmeans 1.11.2-8 (Lenth *et al.*, 2025), and car 3.1-2 (Fox & Weisberg, 2019). Data were visualized using ggplot2 (Wickham, 2016) and RColorBrewer (Neuwirth, 2022). Data organization aids were tidyr (Wickham *et al.*, 2024) and dplyr (Wickham *et al.*, 2023).

We calculated larval growth rate after 24 h using the formula (lm24 – lm0)/lm0, where lm24 is the average larval mass after 24 h. Weight change of adults during the duration of post-hatching care was calculated in a similar manner. To analyze the effects of the treatments on larvae and adults, we used generalized linear models (GLM), all fitted with a Gaussian distribution if not stated oth-

erwise. Survival of larvae was first transformed using a Yeo–Johnson transformation, as this allows for handling negative values and zero, to ensure model fit and homoscedasticity. Survival was then analyzed using a GLM (survival ~ carcass age $\times$ care pattern). A GLM was used to investigate effects of treatments on the growth rate of larvae after 24 h of post-hatching care. Treatments effects of larval PO and total-PO activity were analyzed similarly. Effects on larval hemocyte count were tested for by using a glmmTMB (McGillcuddy *et al.*, 2025), adjusted for zero-inflation and employing a negative binomial distribution.

We investigated immunity and performance of adults after post-hatching care by combining the effects of sex and care patterns (e.g., biparental female care and biparental male care instead of just biparental care) into one factor for analysis, together with carcass age. To ensure model fit and homoscedasticity, $V_{\max}$ values of oral exudates and of PO activity of adult beetles were transformed using a Yeo–Johnson transformation. Testing aspects of adult performance or immunity, we used GLMers that included the sample ID as a random factor (adult performance parameter ~ carcass age $\times$ care pattern based on sex + (1|ID)).

To obtain P -values of general effects, we used an Anova (type III) from the “car” package, and to perform *post hoc* pairwise comparisons we used an emmeans function, adjusted with the Tukey’s method. To determine the directions of significant interactions, we used custom contrasts for relevant pairwise comparisons, we used emmeans, adjusted for “false discovery rate.” Model fidelity was controlled with the DHARMA package.

Results

Offspring performance

Larval growth rate Growth rate of larvae during the first 24 h was affected by both the care pattern (P -value < 0.001, $\chi^2_2 = 20.08$, Fig. 1A, Table 1) and the carcass age as well (P -value < 0.001, $\chi^2_1 = 16.24$, Fig. 1A, Table 1) but was not affected by the interaction of both factors (P -value = 0.355, $\chi^2_2 = 2.07$, Fig. 1A, Table 1). *Post hoc* comparisons revealed that larvae which received uniparental male care grew slower than larvae that received uniparental female care or biparental care, while there was no difference between uniparental female and biparental care (Table S1).

Larval survival After 24 h of development in the carcass nursery, larval survival was affected by the carcass

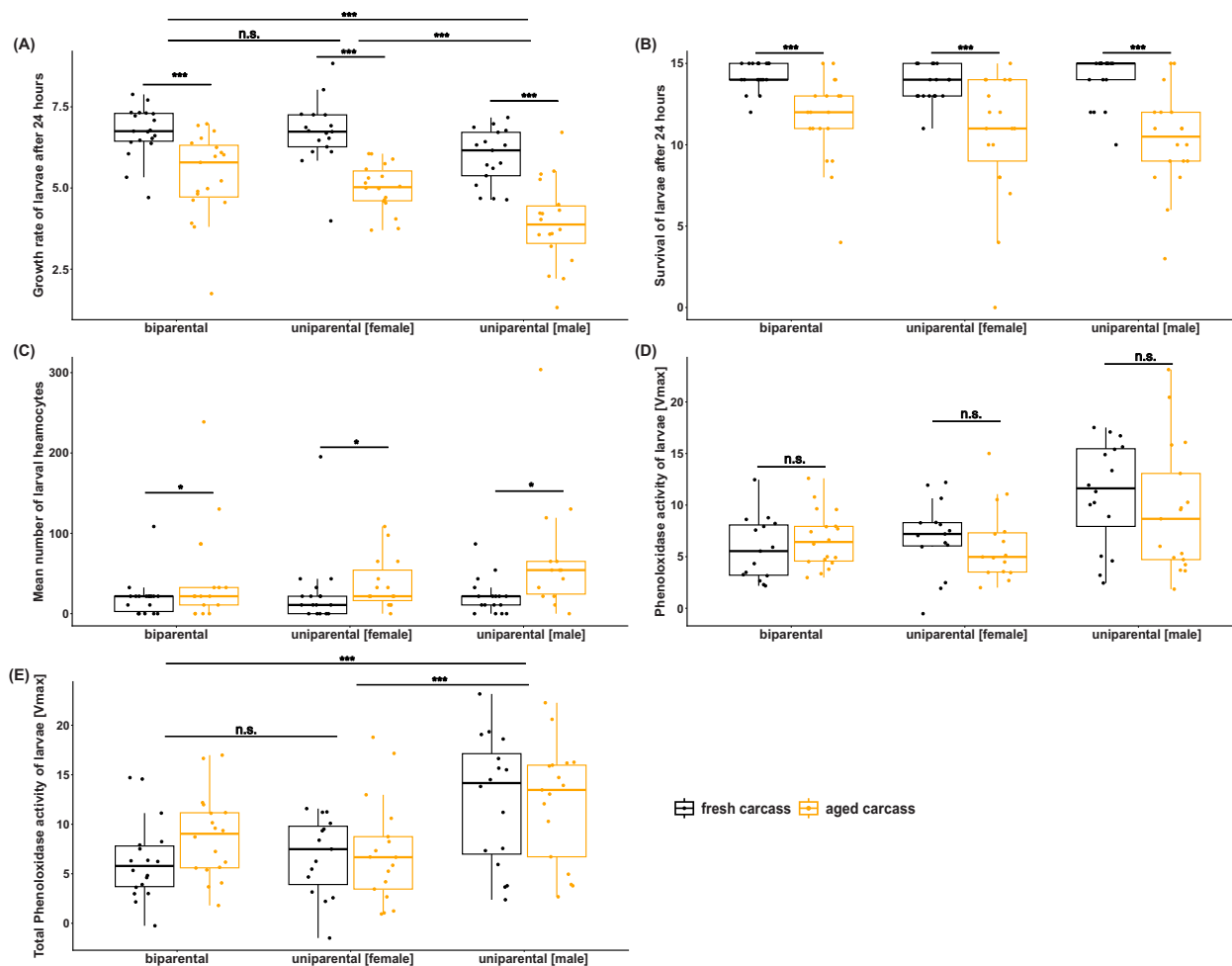


Fig. 1 Boxplots showcasing offspring performance and immunity of *Nicrophorus vespilloides* larvae after 24 h, depending on the carcass age and the care pattern with which larvae developed. (A) Larval growth rate after 24 h. (B) Number of surviving larvae after 24 h. (C) Mean number of hemocytes in larval hemolymph. (D) Phenoloxidase activity of larval homogenates. (E) Total Phenoloxidase activity of larval homogenates. Boxplots show median (horizontal line), 25% and 75% quartile (boxes), and 1.5× interquartile range (whiskers). Statistical differences are indicated by asterisks, * $P < 0.05$; *** $P < 0.001$; n.s., not significant.

age (P -value < 0.001 , $\chi^2_1 = 18.26$, Fig. 1B, Table 1) as more larvae survived on fresh carcasses than on aged ones, but survival was unaffected by care pattern (P -value $= 0.293$, $\chi^2_2 = 2.46$, Fig. 1B, Table 1) or the interaction of both treatments (P -value $= 0.5$, $\chi^2_2 = 1.39$, Fig. 1B, Table 1).

Offspring immunity

Larval hemocytes Number of hemocytes in the larval hemolymph was affected by the carcass age (P -value $= 0.033$, $\chi^2_1 = 4.56$, Fig. 1C, Table 2), showing an increase if larvae developed on an aged carcass. Hemocyte number

was not affected by care pattern (P -value $= 0.232$, $\chi^2_2 = 2.92$, Fig. 1C, Table 2) nor by an interaction between carcass age and care pattern (P -value $= 0.33$, $\chi^2_2 = 2.21$, Fig. 1C, Table 2).

Larval PO activity and total PO activity The PO activity in larval hemolymph was not affected by the carcass age (P -value $= 0.54$, $\chi^2_1 = 0.38$, Fig. 1D, Table 2), care pattern (P -value $= 0.054$, $\chi^2_2 = 5.82$, Fig. 1D, Table 2) nor by their interaction (P -value $= 0.463$, $\chi^2_2 = 1.54$, Fig. 1D, Table 2). However, the total-PO activity was affected by the care pattern (P -value $= 0.013$, $\chi^2_2 = 8.67$, Fig. 1E, Table 2), but not by the carcass age (P -value $= 0.144$, $\chi^2_1 = 2.14$, Fig. 1E, Table 2) or by an interaction

Table 1 Effects of carcass age and care pattern on offspring performance in *N. vespilloides* larvae.

<i>Survival of larvae</i>				
Generalized linear model	Predictor	χ^2	DF	<i>P</i> -value
	Carcass age	18.259	1	< 0.001
	Care pattern	2.456	2	0.293
	Carcass age $\times$ Care pattern	1.386	2	0.500
<i>Growth rate of larvae</i>				
Generalized linear model	Predictor	χ^2	DF	<i>P</i> -value
	Carcass age	16.236	1	< 0.001
	Care pattern	20.082	2	< 0.001
	Carcass age $\times$ Care pattern	2.072	2	0.355

Note: Statistically significant effects are highlighted in bold.

Table 2 Effects of carcass age and care pattern on offspring immunity in *N. vespilloides* larvae.

<i>Larval hemocytes</i>				
Generalized linear mixed model	Predictor	χ^2	DF	<i>P</i> -value
	Carcass age	4.560	1	0.033
	Care pattern	2.918	2	0.232
	Carcass age $\times$ Care pattern	2.210	2	0.331
<i>Larval phenoloxidase activity [V_{max}]</i>				
Generalized linear model	Predictor	χ^2	DF	<i>P</i> -value
	Carcass age	0.375	1	0.540
	Care pattern	5.822	2	0.054
	Carcass age $\times$ Care pattern	1.542	2	0.463
<i>Larval total phenoloxidase activity [V_{max}]</i>				
Generalized linear model	Predictor	χ^2	DF	<i>P</i> -value
	Carcass age	2.135	1	0.144
	Care pattern	8.666	2	0.013
	Carcass age $\times$ Care pattern	1.348	2	0.510

Note: Statistically significant effects are highlighted in bold.

of the two (P -value = 0.51, $\chi^2_2 = 1.35$, Fig. 1E, Table 2). *Post hoc* comparison revealed that larvae that received uniparental male care had an increased total-PO activity than larvae that received biparental or uniparental female care (Table S2). There was no difference in total-PO activity between larvae in the biparental care treatment and the uniparental female care treatment (Table S2).

Adult performance and immunity

Adult oral exudate lytic activity We found no effects of carcass age (P -value = 0.851, $\chi^2_1 = 0.04$, Fig. 2A, Table 3), sex-based care pattern (P -value = 0.446, $\chi^2_2 = 1.61$, Fig. 2A, Table 3), nor their interaction (P -value = 0.813, $\chi^2_2 = 0.42$, Fig. 2A, Table 3) on lytic activity of adult oral exudates.

Adult PO activity During post-hatching care, the PO activity of attending adults was affected by an interaction between the sex-based care pattern and carcass age (P -value = 0.003, $\chi^2_3 = 13.76$, Fig. 2B, Table 3) but not by carcass age (P -value = 0.582, $\chi^2_1 = 0.30$, Table 3) or by the care pattern alone (P -value = 0.066, $\chi^2_3 = 7.20$, Table 3). Additional *post hoc* testing revealed that uniparentally caring females and males showed an increased PO activity if they were caring on an aged carcass compared to post-hatching care on fresh carcasses (Table S4). Interestingly, the PO activity of males and females were not affected by the carcass age during biparental care (Table S4). However, biparentally caring males on a fresh carcass showed higher PO activity than their female partners (Table S4). There was no such difference between biparentally caring males and females on aged carcasses. While PO activity of uniparentally and biparentally car-

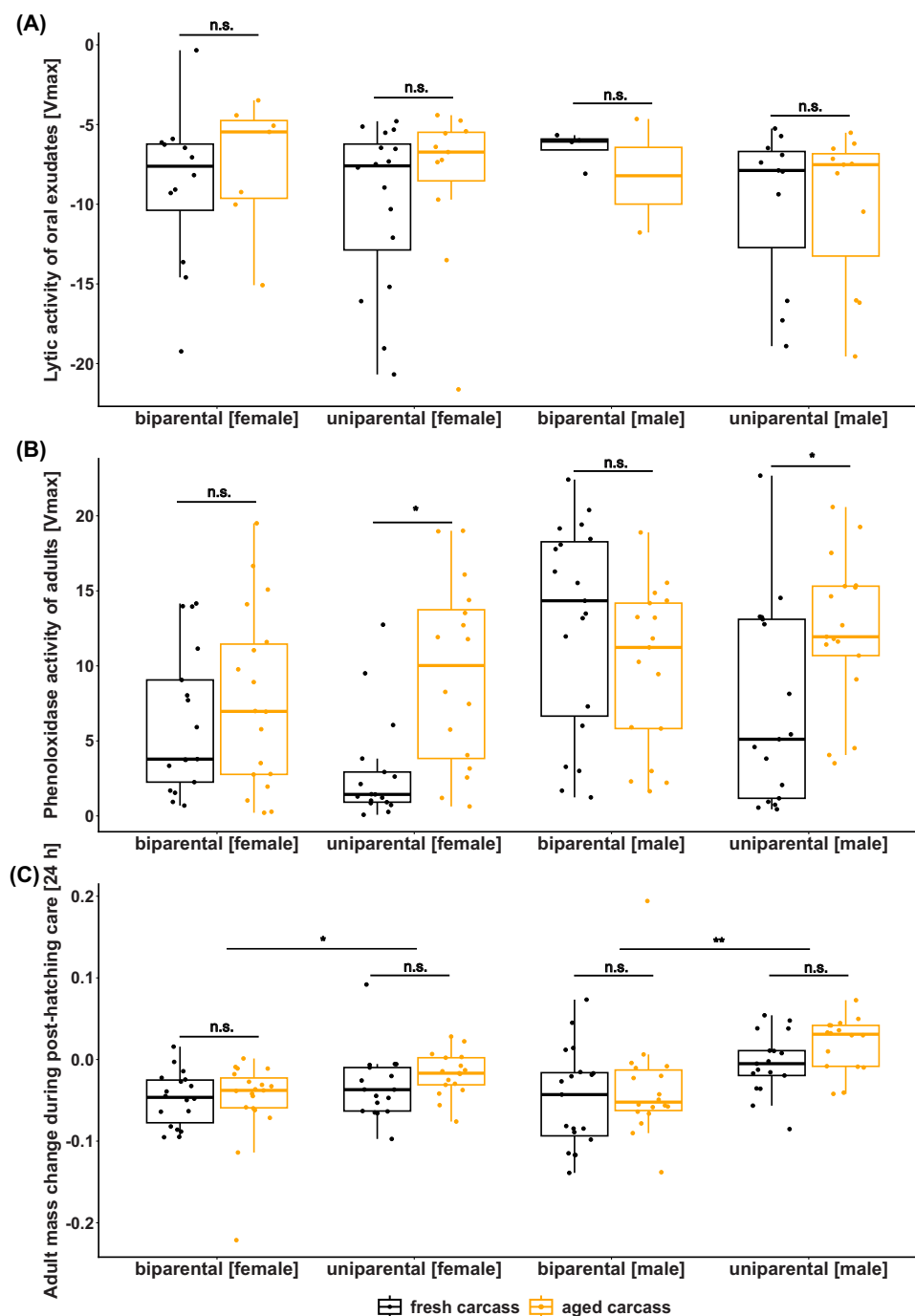


Fig. 2 Boxplots showcasing immunity of *N. vespilloides* adult beetles after 24 h, depending on the carcass age and the care pattern. (A) Lytic activity of adult oral exudates. (B) Phenoloxidase activity of adult hemolymph extracts. (C) Body mass change of adults during the time of care. Boxplots show median (horizontal line), 25% and 75% quartile (boxes), and 1.5 $\times$ interquartile range (whiskers). Statistical differences are indicated by asterisks, * $P < 0.05$; ** $P < 0.01$; n.s., not significant.

Table 3 Effects of carcass age and sex-based care pattern on mass change of *N. vespilloides* adults, as well as their personal and social immunity.

<i>Adult mass change</i>				
Generalized linear mixed model	Predictor	χ^2	DF	P-value
	Carcass age	0.003	1	0.959
	Care pattern split	20.902	3	< 0.001
	Care pattern split $\times$ Carcass age	1.321	3	0.724
<i>Adult phenoloxidase activity [V_{max}]</i>				
Generalized linear mixed model	Predictor	χ^2	DF	P-value
	Carcass age	0.304	1	0.582
	Care pattern	7.198	3	0.066
	Care pattern split $\times$ Carcass age	13.758	3	0.003
<i>Lytic activity of oral exudates [V_{max}]</i>				
Generalized linear mixed model	Predictor	χ^2	DF	P-value
	Carcass age	0.035	1	0.851
	Care pattern	1.613	2	0.446
	Carcass age $\times$ Care pattern	0.415	2	0.813

Note: Statistically significant effects are highlighted in bold.

ing adult beetles did not differ on an aged carcass, their PO activity differed when they are caring on a fresh carcass, as uniparentally caring males and females showed a reduced PO activity (Table S4).

Mass change After 24 h of post-hatching care, mass change of caring adults was influenced by the sex-based care pattern (P -value < 0.001, $\chi^2_3 = 20.90$, Fig. 2C, Table 3) but not by the carcass age (P -value = 0.959, $\chi^2_1 = 0.03$, Fig. 2C, Table 3) or by the interaction of both factors (P -value = 0.724, $\chi^2_3 = 1.32$, Fig. 2C, Table 3). *Post hoc* comparisons revealed that uniparentally caring females lost less weight during the post-hatching care period than biparentally caring females. The same pattern can be observed in caring males (Table S3). While there was no difference in mass change between biparentally caring females and males, uniparentally caring males lost less weight than uniparentally caring females during the 24 h of post-hatching care (Table S3).

Discussion

Biparental care proves to be an evolutionary stable strategy when it is either very efficient for parents, necessary for offspring survival, or doubles offspring fitness (Maynard Smith, 1977; Motro, 1994; Trumbo, 2006; Barta *et al.*, 2014). In this study, we demonstrate benefits of biparental care in a species with facultative uni- and biparental care and high flexibility in parental roles. We also show that larvae benefit more (at least short-term)

from biparental and uniparental female than uniparental male care in terms of growth rate and immunity under adverse environmental conditions, despite previous indications that uniparental male and female care are equivalent for offspring (e.g., Bartlett, 1988; Smith *et al.*, 2017).

It has been demonstrated that *Nicrophorus* males can fully compensate for an absent female, resulting in similar brood size and brood mass (Fetherston *et al.*, 1994; Smiseth *et al.*, 2005). Here, we show that larvae under uniparental male care perform worse during the first 24 h, which may have long-term consequence for offspring fitness (Lock *et al.*, 2004). Specifically, larvae show a reduction in growth rate under uniparental male care and increased investment into their personal immunity through a higher PO activity. PO is an important enzyme in cuticle melanization and immune response of insects, defending the organism from pathogen and parasites whose activity levels are directly related to the immune defense capabilities of the insect (Wilson *et al.*, 2001; González-Santoyo & Córdoba-Aguilar, 2012). We hypothesize that these changes in larval performance and immunity under uniparental male care indicate that males do not fully compensate for their absent partner in terms of direct care (Trumbo, 1991; Fetherston *et al.*, 1994; Smiseth *et al.*, 2005), social immunity and carcass maintenance. Insufficient compensation by males may be due to positive feedback of selection favoring female direct care, given the fact that females are far less likely to desert their brood (McNamara & Wolf, 2015; Fromhage & Jennions, 2016; Trumbo, 2022). *Nicrophorus orbicollis* females in-

vest more into personal immunity (Steiger *et al.*, 2011; Steiger *et al.*, 2012) but whether these immunity differences extend to offspring benefits from social immunity, no less those of other *Nicrophorus* species, is unknown. Ultimately, we do not detect differences in lytic activity of male and female oral exudates that suggest sex-specific social immunity capabilities that would force larvae to invest more into their personal immunity and less into growth, potentially exacerbating insufficient direct care compensation.

Instead, increased immunity investments of offspring might be reflective of task asymmetry of the caring sex. On average, *N. vespilloides* males are prone to abandoning the brood (Müller *et al.*, 2007) and spend less time feeding larvae with regurgitated carcass than females (Körner *et al.*, 2026), an essential part of post-hatching care that strongly boosts offspring performance and survival (Smiseth & Moore, 2004; Capodeanu-Nägler *et al.*, 2016; Capodeanu-Nägler *et al.*, 2018b). Feeding does not only transfer predigested food but also beneficial enzymes, microbes and antimicrobial substances (Shukla *et al.*, 2018b; Körner *et al.*, 2023), which could be essential for larval performance and early immunity. While previous studies show that sex differences of uniparental care in offspring performance do not last until dispersal, our results show that male care does fare worse during the first 24 h of larval development, a time postulated to be a critical phase of *Nicrophorus* family life benefits (Capodeanu-Nägler *et al.*, 2018b). Our findings, however, only allow speculations on the later performance of offspring. While the first 24 h may be critical, larval mass at dispersal determines adult body size after eclosion (Jarrett *et al.*, 2017) and thus directly influences fitness of offspring later in life. As larvae become more and more independent, being fully self-sufficient after 72 h on the carcass (Smiseth *et al.*, 2003), early differences in growth rate and larval body mass might not necessarily persist until larvae leave the carcass (Müller *et al.*, 2025), making predictions about their fitness at a later stage difficult. It also is important to consider that, while uniparentally caring males adjust their behavior and gene expression to compensate for the sudden loss of their partner (Fetherston *et al.*, 1994; Parker *et al.*, 2015), such changes may occur slowly and take time to come into full effect, allowing widowed males to possibly better compensate for the missing female in full at a later point in time. Our results call for further investigations into the long-term effects of increased immune investment during early development and how it shapes adult fitness and later life history traits.

Despite care pattern effects on larval performance and PO activity, larval survival was only affected by car-

cass age: fewer larvae survived on aged carcasses while the number of hemocytes in their hemolymph was increased. Hemocytes are an integral part of the insect's cellular immune response and mediate phagocytosis, wound healing and encapsulation of foreign bodies (Lavine & Strand, 2002; Rolff & Siva-Jothy, 2004; Strand, 2008; Eleftherianos *et al.*, 2021). Increased larval hemocytes on aged carcasses likely represent increased investments into personal immunity, responding to the increased microbial load of the carcass nursery. Notably, this occurred regardless of care pattern, indicating that the attending adults struggle to completely control the aged carcass' microbiome. Older carcasses with a higher microbial load pose a significant challenge for *Nicrophorus* adults, their offspring and following generations alike (Cotter *et al.*, 2010; McLean *et al.*, 2014; Trumbo *et al.*, 2016; Trumbo, 2016a) largely due to the release of toxic metabolites and attraction of competitors through microbial volatiles (Trumbo & Sikes, 2021), requiring a simultaneous investment into social and personal immunity (Rozen *et al.*, 2008; Arce *et al.*, 2012; Arce *et al.*, 2013; Jacobs *et al.*, 2014; Shukla *et al.*, 2018a; Wang & Rozen, 2019; Körner *et al.*, 2023). Under natural conditions *Nicrophorus* spp. monopolize most carcasses 1–4 d after decomposition has started (Wilson & Fudge, 1984; Trumbo, 1990; Trumbo, 2016b) while carcass decomposition at 7 d may be exacerbated in the wild due to the involvement of a more diverse microbiome found in soil of fields and forests (Lauber *et al.*, 2014; Hilal *et al.*, 2021). Interestingly, larval total-PO was affected only by the care larvae received, as it was increased under uniparental male care, while there were no detectable differences between biparental and uniparental female care nor between aged and fresh carcasses. Offspring of *N. vespilloides* thus respond to social and non-social environmental factors by increasing different aspects of their immunity. Past studies showed upregulation of select immune genes in parental absence (Ziadie *et al.*, 2019), and our data indicate that *Nicrophorus* larvae are also selected to adjust their immune expression in response to non-social factors as well. Future studies should investigate which cues *Nicrophorus* offspring use to determine the necessity for their own investment, and to what extent different offspring dependency (Capodeanu-Nägler *et al.*, 2018a) affects these adjustments.

Parental division of labor during biparental care may alleviate the costs of care for each partner (Trumbo, 1991; Cotter & Kilner, 2010b). We therefore expected biparentally caring beetles to show lower social immunity, and that this lower energy expenditure is reflected in overall higher weight compared to uniparentally caring beetles (Trumbo & Xihani, 2015; Wang *et al.*, 2021). Per-

sonal immunity may also be affected by better control of the carcass microbiome. Furthermore, we expected these effects to be exacerbated on aged carcasses. Interestingly, our hypotheses were refuted: while beetles of either sex gained more weight after caring uniparentally compared to biparentally, they showed no adjustment to the lytic activity of their oral exudates. Neither effect was dependent on the carcass age. In *N. orbicollis*, breeding beetles gain weight during pre-hatching care, with the amount of gained weight depending on the size of the carcass resource, and then rapidly lose weight during the time of parental care (Trumbo & Rauter, 2014). In our study, uniparentally caring *N. vespilloides* females and males may choose to withhold resources and not invest as much into their current generation of offspring in an effort to save energy for a later reproductive event (reproductive restraint; McNamara *et al.*, 2009) where the costs of post-hatching care can be shared with a partner, as resource allocation between current and future reproductive bouts must be carefully balanced (Creighton *et al.*, 2009; Wang *et al.*, 2022). Females may benefit from early male desertion, as now freed-up resources can be used to offset some of the costs mating and parental care have incurred (Boncoraglio & Kilner, 2012). While both parents gain fitness benefits through the shared care for their offspring, and adjust their own food intake based on their own body size and on how much carrion their partner consumes (Pilakouta *et al.*, 2016), males might specifically stay to secure a valuable resource, increasing their own fitness and attractiveness for future reproductive events (Chemnitz *et al.*, 2017). Beetles in our experiment just recently sexually matured and may therefore have reserved resources for a reproductive event under better environmental conditions (McNamara *et al.*, 2009).

Surprisingly, our data also show that advanced stages of decay do not elicit a stronger social immune response than fresh carcasses in the form of increased lytic activity of oral exudates. As *Nicrophorus* beetles are competing with the carcass-associated microbiome over the carrion resource, they have evolved an effective mechanism to counteract the continuous decay. Their anal and oral exudates are capable of attenuating the growth of fungi and bacteria and their production is increased when beetles come across a carcass (Rana *et al.*, 1997; Suzuki, 2001; Hoback *et al.*, 2004; Cotter & Kilner, 2010b). Due to its pivotal role in niche construction for *Nicrophorus* (Arce *et al.*, 2012; Körner *et al.*, 2023; Potticary *et al.*, 2024), it is possible that parental social immunity is always expressed at peak capacity during early post-hatching care

which would explain our results. Alternatively, it is possible that exudate lytic activity is only increased during early carcass preparation and had already returned to usual levels at the time of our measurement. Future studies should take this into account when investigating the social immunity response as part of the pre-hatching care of *Nicrophorus* species.

Conversely, we found that beetles did adjust their personal immunity to both care pattern and carcass age, but again not quite as expected. Uniparentally caring beetles on aged carcasses had an overall higher PO activity than uniparentally caring beetles on fresh carcasses, regardless of sex. Increased personal immunity is an expected effect of higher microbial prevalence: as single parents struggle to control the carcass microbiome they are subjected to its microbes and forced to invest more in their own protection (Cotter *et al.*, 2010; Cotter *et al.*, 2013). Upregulation in immunity following a breeding event and sex differences in immunity have been shown in this genus before (Steiger *et al.*, 2011), but the pattern in PO activity of uniparentally caring beetles, we observe, is very intriguing. Assuming that biparental care on a fresh carcass is the norm, we find that uniparentally caring females and males show a reduced PO activity compared to their biparentally caring counterparts. This effect is reversed under adverse environmental conditions, for example, an aged carcass, as uniparentally caring beetles increase their PO activity under such circumstances, potentially causing them to favor reproductive restraint, increasing their chances of survival until the next reproductive event. Interestingly, we observe no adjustment in PO activity toward the microbial environment when beetles are caring together with a partner. This might suggest that the shared workload during biparental care allows *Nicrophorus* beetles to offset harmful environmental conditions at reduced energy costs for the individual. However, increased immunity alone is difficult to interpret, as it can also incur costs for life-history traits (Zuk & Stoehr, 2002; Ardia *et al.*, 2012; Leyria *et al.*, 2025). Overall, however, our results emphasize that biparental care may allow adults to maintain immune function under challenging conditions while sustaining offspring performance.

Pilakouta *et al.* (2018) demonstrated synergistic effects of biparental care in burying beetles by standardizing per-parent workload. In our study, we specifically did not manipulate per-parent workload or resource availability to determine negative effects of uniparental care that may be compensated by caring biparentally, both for the adult beetles and their offspring, in terms of immunity and performance. We present novel evidence that biparental care

and female uniparental care appear to be equally advantageous in terms of development and immunity for the offspring, while male uniparental care comes with costs for the offspring. We did not find any clear evidence indicating that biparental care is beneficial to both the female and the male parent in terms of reduced energy investment due to a shared workload, which would be noticeable in their weight change during post-hatching care. However, our data suggest that biparental care may allow parents to reserve more energy for their own immune system compared to individuals who care for their offspring alone. Still, direct benefits of biparental care remain ambiguous as an increased immune activity can be a double-edged sword, which can prove to be costly for the organism (Zdybicka-Barabas *et al.*, 2025). We also provide first evidence that larval immunity is highly dependent on the quality of the carcass resource, and the kind of parental care larvae receive. Our results thus show that uniparental care of *N. vespilloides* is not equivalent to biparental care, and that single males do not care as well as females, as previously proposed. This opens further questions about how parental care patterns and microbial environment affect immune regulatory and developmental processes in *Nicrophorus* offspring.

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Disclosure

The authors have no conflict of interest to declare.

References

- Adams, E.M., Jones, A.G. and Arnold, S.J. (2005) Multiple paternity in a natural population of a salamander with long-term sperm storage. *Molecular Ecology*, 14, 1803–1810.
- Arce, A.N., Johnston, P.R., Smiseth, P.T. and Rozen, D.E. (2012) Mechanisms and fitness effects of antibacterial defences in a carrion beetle. *Journal of Evolutionary Biology*, 25, 930–937.
- Arce, A.N., Smiseth, P.T. and Rozen, D.E. (2013) Antimicrobial secretions and social immunity in larval burying beetles, *Nicrophorus vespilloides*. *Animal Behaviour*, 86, 741–745.
- Ardia, D.R., Gantz, J.E., Schneider, B.C. and Strebel, S. (2012) Costs of immunity in insects: an induced immune response increases metabolic rate and decreases antimicrobial activity. *Functional Ecology*, 26, 732–739.
- Barta, Z., Székely, T., Liker, A. and Harrison, F. (2014) Social role specialization promotes cooperation between parents. *The American Naturalist*, 183, 747–761.
- Bartlett, J. (1988) Male mating success and paternal care in *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Behavioral Ecology and Sociobiology*, 23, 297–303.
- Bartlett, J. and Ashworth, C.M. (1988) Brood size and fitness in *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Behavioral Ecology and Sociobiology*, 22, 429–434.
- Bates, D., Mächler, M., Bolker, B. and Walker, S. (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67, 1.
- Benowitz, K.M. and Moore, A.J. (2016) Biparental care is predominant and beneficial to parents in the burying beetle *Nicrophorus orbicollis* (Coleoptera: Silphidae). *Biological Journal of the Linnean Society*, 119, 1082–1088.
- Boncoraglio, G. and Kilner, R.M. (2012) Female burying beetles benefit from male desertion: sexual conflict and counter-adaptation over parental investment. *PLoS ONE*, 7, e31713.
- Capodeanu-Nägler, A., Eggert, A.K., Vogel, H., Sakaluk, S.K. and Steiger, S. (2018a) Species divergence in offspring begging and parental provisioning is linked to nutritional dependency. *Behavioral Ecology*, 29, 42–50.
- Capodeanu-Nägler, A., Keppner, E.M., Vogel, H., Ayasse, M., Eggert, A.-K., Sakaluk, S.K. *et al.* (2016) From facultative to obligatory parental care: interspecific variation in offspring dependency on post-hatching care in burying beetles. *Scientific Reports*, 6, 29323.
- Capodeanu-Nägler, A., Prang, M.A., Trumbo, S.T., Vogel, H., Eggert, A.-K., Sakaluk, S.K. *et al.* (2018b) Offspring dependence on parental care and the role of parental transfer of oral fluids in burying beetles. *Frontiers of Zoology*, 15, 33.
- Cerenius, L. and Söderhäll, K. (2004) The prophenoloxidase-activating system in invertebrates. *Immunological Reviews*, 198, 116–126.
- Chemnitz, J., Bagrii, N., Ayasse, M. and Steiger, S. (2017) Staying with the young enhances the fathers' attractiveness in burying beetles. *Evolution; International Journal of Organic Evolution*, 71, 985–994.
- Cockburn, A. (2006) Prevalence of different modes of parental care in birds. *Proceedings of the Royal Society B: Biological Sciences*, 273, 1375–1383.
- Cotter, S.C. and Kilner, R.M. (2010a) Personal immunity versus social immunity. *Behavioral Ecology*, 21, 663–668.

- Cotter, S.C. and Kilner, R.M. (2010b) Sexual division of antibacterial resource defence in breeding burying beetles, *Nicrophorus vespilloides*. *Journal of Animal Ecology*, 79, 35–43.
- Cotter, S.C., Littlefair, J.E., Grantham, P.J. and Kilner, R.M. (2013) A direct physiological trade-off between personal and social immunity. *Journal of Animal Ecology*, 82, 846–853.
- Cotter, S.C., Topham, E., Price, A.J.P. and Kilner, R.M. (2010) Fitness costs associated with mounting a social immune response. *Ecology Letters*, 13, 1114–1123.
- Creighton, J.C., Heflin, N.D. and Belk, M.C. (2009) Cost of reproduction, resource quality, and terminal investment in a burying beetle. *The American Naturalist*, 174, 673–684.
- Creighton, J.C., Smith, A.N., Komendat, A. and Belk, M.C. (2015) Dynamics of biparental care in a burying beetle: experimental handicapping results in partner compensation. *Behavioral Ecology and Sociobiology*, 69, 265–271.
- Cremer, S., Armitage, S.A.O. and Schmid-Hempel, P. (2007) Social immunity. *Current Biology*, 17, R693–R702.
- Cremer, S., Pull, C.D. and Fürst, M.A. (2018) Social immunity: emergence and evolution of colony-level disease protection. *Annual Review of Entomology*, 63, 105–123.
- Degenkolb, T., Düring, R.A. and Vilcinskas, A. (2011) Secondary metabolites released by the burying beetle *Nicrophorus vespilloides*: Chemical analyses and possible ecological functions. *Journal of Chemical Ecology*, 37, 724–735.
- Eggert, A.K. (1992) Alternative male mate-finding tactics in burying beetles. *Behavioral Ecology*, 3, 243–254.
- Eggert, A.K. and Müller, J.K. (2011) Timing of oviposition enables dominant female burying beetles to destroy brood-parasitic young. *Animal Behaviour*, 82, 1227–1233.
- Eleftherianos, I., Heryanto, C., Bassal, T., Zhang, W., Tettamanti, G. and Mohamed, A. (2021) Haemocyte-mediated immunity in insects: cells, processes and associated components in the fight against pathogens and parasites. *Immunology*, 164, 401–432.
- Fetherston, I.A., Scott, M.P. and Traniello, J.F.A. (1994) Behavioural compensation for mate loss in the burying beetle *Nicrophorus orbicollis*. *Animal Behaviour*, 47, 777–785.
- Fox, J. and Weisberg, S. (2019) *An R Companion to Applied Regression*. Sage, CA, USA.
- Fromhage, L. and Jennions, M.D. (2016) Coevolution of parental investment and sexually selected traits drives sex-role divergence. *Nature Communications*, 7, 12517.
- Ginsberg, J.R. and Huck, U.W. (1989) Sperm competition in mammals. *Trends in Ecology & Evolution*, 4, 74–79.
- González-Santoyo, I. and Córdoba-Aguilar, A. (2012) Phenoloxidase: a key component of the insect immune system. *Entomologia Experimentalis et Applicata*, 142, 1–16.
- Grafen, A. (1990) Biological signals as handicaps. *Journal of Theoretical Biology*, 144, 517–546.
- Grew, R., Ratz, T., Richardson, J. and Smiseth, P.T. (2019) Parental care buffers against effects of ambient temperature on offspring performance in an insect. *Behavioral Ecology*, 30, 1443–1450.
- Harrison, F., Barta, Z., Cuthill, I. and Székely, T. (2009) How is sexual conflict over parental care resolved? A meta-analysis. *Journal of Evolutionary Biology*, 22, 1800–1812.
- Hartig, F. (2022) DHARMA: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models. <https://cran.r-project.org/web/packages/DHARMA/vignettes/DHARMA.html>.
- He, S., Johnston, P.R., Kuropka, B., Lokatis, S., Weise, C., Plarre, R. et al. (2018) Termite soldiers contribute to social immunity by synthesizing potent oral secretions. *Insect Molecular Biology*, 27, 564–576.
- Hilal, M.G., Yu, Q., Zhou, R., Wang, Y., Feng, T., Li, X. et al. (2021) Exploring microbial communities, assessment methodologies and applications of animal's carcass decomposition: a review. *FEMS Microbiology Ecology*, 97, fiab098.
- Hoback, W.W., Bishop, A.A., Kroemer, J., Scalzitti, J. and Shaffer, J.J. (2004) Differences among antimicrobial properties of carrion beetle secretions reflect phylogeny and ecology. *Journal of Chemical Ecology*, 30, 719–729.
- Houston, A.I. and Davies, N.B. (1985) The evolution of cooperation and life history in the dunnoek *Prunella modularis*. In *Behavioural Ecology: Ecological Consequences of Adaptive Behaviour* (eds. R.M. Sibly & R.H. Smith), pp. 471–487. Blackwell Scientific Publications, Oxford.
- Jacobs, C.G.C., Wang, Y., Vogel, H., Vilcinskas, A., van der Zee, M. and Rozen, D.E. (2014) Egg survival is reduced by grave-soil microbes in the carrion beetle, *Nicrophorus vespilloides*. *BMC Evolutionary Biology*, 14, 208.
- Janzen, D.H. (1977) Why fruits rot, seeds mold, and meat spoils. *The American Naturalist*, 111, 691–713.
- Jarrett, B.J.M., Schrader, M., Rebar, D., Houslay, T.M. and Kilner, R.M. (2017) Cooperative interactions within the family enhance the capacity for evolutionary change in body size. *Nature Ecology & Evolution*, 1, 0178.
- Johnstone, R.A. and Hinde, C.A. (2006) Negotiation over offspring care—how should parents respond to each other's efforts? *Behavioral Ecology*, 17, 818–827.
- Johnstone, R.A., Manica, A., Fayet, A.L., Stoddard, M.C., Rodríguez-Gironés, M.A. and Hinde, C.A. (2014) Reciprocity and conditional cooperation between great tit parents. *Behavioral Ecology*, 25, 216–222.
- Jones, B., Shipley, E. and Arnold, K.E. (2018) Social immunity in honeybees—density dependence, diet, and body mass trade-offs. *Ecology and Evolution*, 8, 4852–4859.
- Kokko, H. and Jennions, M.D. (2012) Sex differences in parental care. In *The Evolution of Parental Care* (eds. N.J. Royle, P.T. Smiseth & M. Kölliker), pp. 101–112. Oxford University Press, USA.

- Kölliker, M. (2007) Benefits and costs of earwig (*Forficula auricularia*) family life. *Behavioral Ecology and Sociobiology*, 61, 1489–1497.
- Körner, M., Steiger, S. and Shukla, S.P. (2023) Microbial management as a driver of parental care and family aggregations in carrion feeding insects. *Frontiers in Ecology and Evolution*, 11, 1252876.
- Körner, M., Zywucki, L., Reinbold, M. and Steiger, S. (2026) Repeatable differences in male care and sexual conflict resolution during biparental care in a subsocial insect. *Animal Behaviour*, 234, 123520.
- Lauber, C.L., Metcalf, J.L., Keepers, K., Ackermann, G., Carter, D.O. and Knight, R. (2014) Vertebrate decomposition is accelerated by soil microbes. *Applied and Environmental Microbiology*, 80, 4920–4929.
- Lavine, M.D. and Strand, M.R. (2002) Insect hemocytes and their role in immunity. *Insect Biochemistry and Molecular Biology*, 32, 1295–1309.
- Lenth, R.V., Piaskowski, J., Banfai, B., Bolker, B., Buerkner, P., Giné-Vázquez, I. et al. (2025) emmeans: Estimated Marginal Means, aka Least-Squares Means. <https://doi.org/10.32614/CRAN.package.emmeans>.
- Lessells, C.M. and McNamara, J.M. (2011) Sexual conflict over parental investment in repeated bouts: negotiation reduces overall care. *Proceedings of the Royal Society B: Biological Sciences*, 279, 1506–1514.
- Leyria, J., Fruttero, L.L., Paglione, P.A. and Canavoso, L.E. (2025) How insects balance reproductive output and immune investment. *Insects*, 16, 311.
- Lock, J.E., Smiseth, P.T. and Moore, A.J. (2004) Selection, inheritance, and the evolution of parent-offspring interactions. *The American Naturalist*, 164, 13–24.
- Maynard Smith, J. (1977) Parental investment: a prospective analysis. *Animal Behaviour*, 25, 1–9.
- McGillcuddy, M., Warton, D.I., Popovic, G., and Bolker, B.M. (2025) Parsimoniously fitting large multivariate random effects in glmmTMB. *Journal of Statistical Software*, 112, 1–19.
- McLean, A.H.C., Arce, A.N., Smiseth, P.T. and Rozen, D.E. (2014) Late-life and intergenerational effects of larval exposure to microbial competitors in the burying beetle *Nicrophorus vespilloides*. *Journal of Evolutionary Biology*, 27, 1205–1216.
- McNamara, J.M., Houston, A.I., Barta, Z., and Osorno, J.L. (2003) Should young ever be better off with one parent than with two? *Behavioral Ecology*, 14, 301–310.
- McNamara, J.M., Houston, A.I., Barta, Z., Scheuerlein, A. and Fromhage, L. (2009) Deterioration, death and the evolution of reproductive restraint in late life. *Proceedings of the Royal Society B: Biological Sciences*, 276, 4061–4066.
- McNamara, J.M. and Wolf, M. (2015) Sexual conflict over parental care promotes the evolution of sex differences in care and the ability to care. *Proceedings of the Royal Society B: Biological Sciences*, 282, 20142752.
- Meunier, J. (2015) Social immunity and the evolution of group living in insects. *Philosophical Transactions of the Royal Society B*, 370, 20140102.
- Michaut, R.J., Dechambre, R.P., Doumerc, S., Lesourd, B., DeVillechabrolle, A. and Moulias, R. (1981) Influence of early maternal deprivation on adult humoral immune response in mice. *Physiology & Behavior*, 26, 189–191.
- Motro, U. (1994) Evolutionary and continuous stability in asymmetric games with continuous strategy sets: the parental investment conflict as an example. *The American Naturalist*, 144, 229–241.
- Müller, J.K., Braunisch, V., Hwang, W. and Eggert, A.K. (2007) Alternative tactics and individual reproductive success in natural associations of the burying beetle, *Nicrophorus vespilloides*. *Behavioral Ecology*, 18, 196–203.
- Müller, J.K. and 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.
- Müller, J.K., Eggert, A.K. and Fulkröger, E. (1990) Clutch size regulation in the burying beetle *Nicrophorus vespilloides* Herbst (Coleoptera: Silphidae). *Journal of Insect Behavior*, 3, 265–270.
- Müller, L., Steiger, S. and Körner, M. (2025) Surviving in the fast lane: no increased mortality, but faster growth for pathogen-exposed larvae of a family-living beetle. *Journal of Evolutionary Biology*, 38, 1127–1142.
- Neuwirth, E. (2022) RColorBrewer: ColorBrewer Palettes. <https://doi.org/10.32614/CRAN.package.RColorBrewer>.
- Parker, D.J., Cunningham, C.B., Walling, C.A., Stamper, C.E., Head, M.L., Roy-Zokan, E.M. et al. (2015) Transcriptomes of parents identify parenting strategies and sexual conflict in a subsocial beetle. *Nature Communications*, 6, 8449.
- Parker, G.A. (2006) Sexual conflict over mating and fertilization: an overview. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 361, 235–259.
- Pilakouta, N., Hanlon, E.J.H. and Smiseth, P.T. (2018) Biparental care is more than the sum of its parts: experimental evidence for synergistic effects on offspring fitness. *Proceedings of the Royal Society B: Biological Sciences*, 285, 20180875.
- Pilakouta, N., Richardson, J. and Smiseth, P.T. (2016) If you eat, I eat: resolution of sexual conflict over consumption from a shared resource. *Animal Behaviour*, 111, 175–180.
- Posit team (2024) *RStudio: Integrated Development Environment for R*. Posit Software, PBC, Boston, MA, USA.
- Potticary, A.L., Belk, M.C., Creighton, J.C., Ito, M., Kilner, R., Komdeur, J. et al. (2024) Revisiting the ecology and evolution of burying beetle behavior (Staphylinidae: Silphinae). *Ecology and Evolution*, 14, e70175.

- Prang, M.A., Zywucki, L., Körner, M. and Steiger, S. (2022) Differences in sibling cooperation in presence and absence of parental care in a genus with interspecific variation in offspring dependence. *Evolution; International Journal of Organic Evolution*, 76, 320–331.
- R Core Team (2022) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Rana, R.L., Hoback, W.W., Rahim, N.A.A., Bedick, J. and Stanley, D.W. (1997) Pre-oral digestion: a phospholipase A2 associated with oral secretions in adult burying beetles, *Nicrophorus marginatus*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 118, 375–380.
- Ratz, T. (2025) Burying beetles as a model organism to study sex differences in parental care. *Insectes Sociaux*, 72, 193–204.
- Reavey, C.E., Beare, L. and Cotter, S.C. (2014) Parental care influences social immunity in burying beetle larvae. *Ecological Entomology*, 39, 395–398.
- Rolff, J. and Siva-Jothy, M.T. (2004) Selection on insect immunity in the wild. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 271, 2157–2160.
- Rozen, D.E., Engelmoer, D.J.P. and Smiseth, P.T. (2008) Antimicrobial strategies in burying beetles breeding on carrion. *Proceedings of the National Academy of Sciences USA*, 105, 17890–17895.
- Scott, M.P. (1990) Brood guarding and the evolution of male parental care in burying beetles. *Behavioral Ecology and Sociobiology*, 26, 31–39.
- Shukla, S.P., Plata, C., Reichelt, M., Steiger, S., Heckel, D.G., Kaltenpoth, M. *et al.* (2018a) Microbiome-assisted carrion preservation aids larval development in a burying beetle. *Proceedings of the National Academy of Sciences USA*, 115, 11274–11279.
- Shukla, S.P., Vogel, H., Heckel, D.G., Vilcinskis, A. and Kaltenpoth, M. (2018b) Burying beetles regulate the microbiome of carcasses and use it to transmit a core microbiota to their offspring. *Molecular Ecology*, 27, 1980–1991.
- Smiseth, P.T., Darwell, C.T. and Moore, A.J. (2003) Partial begging: an empirical model for the early evolution of offspring signalling. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270, 1773–1777.
- Smiseth, P.T., Dawson, C., Varley, E. and Moore, A.J. (2005) How do caring parents respond to mate loss? Differential response by males and females. *Animal Behaviour*, 69, 551–559.
- Smiseth, P.T., Kolliker, M. and Royle, N.J. (2012) What is parental care? In *The Evolution of Parental Care* (eds N.J. Royle, P.T. Smiseth & M. Kolliker), pp. 1–17. Oxford University Press, USA.
- Smiseth, P.T. and Moore, A.J. (2004) Behavioral dynamics between caring males and females in a beetle with facultative biparental care. *Behavioral Ecology*, 15, 621–628.
- Smith, A.N., Creighton, J.C. and Belk, M.C. (2017) Why does it take two to tango? Lifetime fitness consequences of parental care in a burying beetle. *PLoS ONE*, 12, e0186466.
- Søraker, J.S., Wright, J., Hanslin, F.Ø. and Pepke, M.L. (2023) The evolution of extra-pair paternity and paternal care in birds. *Behavioral Ecology*, 34, 780–789.
- Steiger, S., Gershman, S.N., Pettinger, A.M., Eggert, A.K. and Sakaluk, S.K. (2011) Sex differences in immunity and rapid upregulation of immune defence during parental care in the burying beetle, *Nicrophorus orbicollis*. *Functional Ecology*, 25, 1368–1378.
- Steiger, S., Gershman, S.N., Pettinger, A.M., Eggert, A.K. and Sakaluk, S.K. (2012) Dominance status and sex influence nutritional state and immunity in burying beetles *Nicrophorus orbicollis*. *Behavioral Ecology*, 23, 1126–1132.
- Strand, M.R. (2008) The insect cellular immune response. *Insect Science*, 15, 1–14.
- Suzuki, S. (2001) Suppression of fungal development on carcasses by the burying beetle *Nicrophorus quadripunctatus* (Coleoptera: Silphidae). *Entomological Science*, 4, 403–405.
- Székely, T. and Cuthill, I.C. (1999) Brood desertion in Kentish plover: the value of parental care. *Behavioral Ecology*, 10, 191–197.
- Trivers, R.L. (1972) Parental investment and sexual selection. In *Sexual Selection and the Descent of Man 1871–1971* (ed. B. Campbell), pp. 136–207. Angeles Aldine Publishing Company, Chicago, USA.
- Trumbo, S.T. (1990) Reproductive success, phenology and biogeography of burying beetles (Silphidae, Nicrophorus). *The American Midland Naturalist*, 124, 1–11.
- Trumbo, S.T. (1991) Reproductive benefits and the duration of paternal care in a biparental burying beetle, *Nicrophorus orbicollis*. *Behaviour*, 117, 82–105.
- Trumbo, S.T. (2006) Infanticide, sexual selection and task specialization in a biparental burying beetle. *Animal Behaviour*, 72, 1159–1167.
- Trumbo, S.T. (2007) Defending young biparentally: female risk-taking with and without a male in the burying beetle, *Nicrophorus pustulatus*. *Behavioral Ecology and Sociobiology*, 61, 1717–1723.
- Trumbo, S.T. (2016a) Carcass age and reproductive costs for *Nicrophorus orbicollis* (Coleoptera: Silphidae). *Environmental Entomology*, 45, 1178–1183.
- Trumbo, S.T. (2016b) Fate of mouse carcasses in a Northern Woodland. *Ecological Entomology*, 41, 737–740.
- Trumbo, S.T. (2022) Contrasting sex roles in two species with stable biparental care. *Ethology*, 128, 622–631.

- Trumbo, S.T. and Rauter, C.M. (2014) Juvenile hormone, metabolic rate, body mass and longevity costs in parenting burying beetles. *Animal Behaviour*, 92, 203–211.
- Trumbo, S.T. and Sikes, D.S. (2021) Resource concealment and the evolution of parental care in burying beetles. *Journal of Zoology*, 315, 175–182.
- Trumbo, S.T., Sikes, D.S. and Philbrick, P.K.B. (2016) Parental care and competition with microbes in carrion beetles: a study of ecological adaptation. *Animal Behaviour*, 118, 47–54.
- Trumbo, S.T. and Xihani, E. (2015) Influences of parental care and food deprivation on regulation of body mass in a burying beetle. *Ethology*, 121, 985–993.
- Uller, T. and Olsson, M. (2008) Multiple paternity in reptiles: patterns and processes. *Molecular Ecology*, 17, 2566–2580.
- Van Meyel, S., Körner, M. and Meunier, J. (2018) Social immunity: why we should study its nature, evolution and functions across all social systems. *Current Opinion in Insect Science*, 28, 1–7.
- von Hoersten, S., Dimitrijević, M., Marković, B.M. and Janković, B.D. (1993) Effect of early experience on behavior and immune response in the rat. *Physiology & Behavior*, 54, 931–940.
- Walling, C.A., Stamper, C.E., Smiseth, P.T. and Moore, A.J. (2008) The quantitative genetics of sex differences in parenting. *Proceedings of the National Academy of Sciences USA*, 105, 18430–18435.
- Wang, W., Ma, L., Versteegh, M.A., Wu, H. and Komdeur, J. (2022) Detection of reproductive trade-offs is influenced by resource availability and maintenance: an experimental study in the burying beetle (*Nicrophorus vespilloides*). *Behavioral Ecology and Sociobiology*, 76, 76.
- Wang, W., Ma, L., Versteegh, M.A., Wu, H. and Komdeur, J. (2021) Parental care system and brood size drive sex difference in reproductive allocation: an experimental study on burying beetles. *Frontiers in Ecology and Evolution*, 9, 739396.
- Wang, Y. and Rozen, D.E. (2019) Fitness costs of phoretic nematodes in the burying beetle, *Nicrophorus vespilloides*. *Ecology and Evolution*, 9, 26–35.
- Wickham, H. (2016) *ggplot2: Elegant Graphics for Data Analysis*. Springer, New York. <https://ggplot2.tidyverse.org/>.
- Wickham, H., François, R., Henry, L., Müller, K. and Vaughan, D. (2023) *dplyr: A Grammar of Data Manipulation*. <https://dplyr.tidyverse.org>.
- Wickham, H., Vaughan, D. and Girlich, M. (2024) *tidyr: Tidy Messy Data*. <https://doi.org/10.32614/CRAN.package.tidyr>.
- Wilson, D.S. and Fudge, J. (1984) Burying beetles: intraspecific interactions and reproductive success in the field. *Ecological Entomology*, 9, 195–203.
- Wilson, K., Cotter, S.C., Reeson, A.F. and Pell, J.K. (2001) Melanism and disease resistance in insects. *Ecology Letters*, 4, 637–649.
- Yamamura, N. and Tsuji, N. (1993) Parental care as a game. *Journal of Evolutionary Biology*, 6, 103–127.
- Zdybicka-Barabas, A., Stączek, S., Kunat-Budzyńska, M. and Cytryńska, M. (2025) Innate immunity in insects: the lights and shadows of phenoloxidase system activation. *International Journal of Molecular Sciences*, 26, 1320.
- Ziadie, M.A., Ebot-Ojong, F., McKinney, E.C. and Moore, A.J. (2019) Evolution of personal and social immunity in the context of parental care. *The American Naturalist*, 193, 296–308.
- Zuk, M. and Stoehr, A.M. (2002) Immune defense and host life history. *The American Naturalist*, 160, S9–S22.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Estimated marginal means of the growth of rate of *Nicrophorus vespilloides* larvae for the care pattern larvae received during post-hatching care.

Table S2 Estimated marginal means of the total- phenoloxidase (PO) activity of *N. vespilloides* larvae for the care pattern larvae received during post-hatching care.

Table S3 Custom contrasts of estimated marginal means for the mass change of *N. vespilloides* adults based on the care pattern during post-hatching care.

Table S4 Custom contrasts of estimated marginal means for the PO activity of *N. vespilloides* adults based on the interaction of care pattern and the carcass age during post-hatching care.