ORIGINAL ARTICLE

doi:10.1111/evo.14414

Differences in sibling cooperation in presence and absence of parental care in a genus with interspecific variation in offspring dependence

Madlen A. Prang,[1,2] **Lena Zywucki,**[1] **Maximilian Körner,**[1] **and Sandra Steiger**[1]

[1] *Department of Evolutionary Animal Ecology, University of Bayreuth, Bayreuth DE-95440, Germany*

[2] *E-mail: prang.madlen@gmail.com*

Received January 12, 2021
Accepted November 4, 2021

The widely spread evolutionary strategy of parental care is considered an important driver of social evolution. Although offspring were long thought to primarily interact competitively, recent studies revealed the potential importance of sibling cooperation. Theories suggest that the degree of cooperation in offspring interactions depends on the degree of offspring dependence on parental care: offspring unable to forage on their own should compete more, whereas more independent juveniles may increase the degree of cooperation. In this study, we tested the occurrence of sibling cooperation in the absence of posthatching care in several burying beetle species exhibiting varying degrees of offspring dependence. To this end, we measured larval growth rate and survival in the presence and absence of prehatching care using different brood sizes. We found that sibling cooperation cannot be exclusively explained by offspring dependence on parental care. Although only species with more independent larvae cooperated when receiving prehatching care, larval cooperation occurred across species in the absence of care. The latter result suggests that sibling cooperation was already present in an early ancestor of the genus *Nicrophorus*. Overall, these findings give important insights into the transition from facultative to obligate family life.

KEY WORDS: Burying beetle, family life, *Nicrophorus*, offspring dependence, parental care, social evolution.

The taxonomically widespread phenomenon of family life, that is, the association of offspring with their parents after birth or hatching (Kramer and Meunier 2019), is considered an important first step in the evolution of sociality (Clutton-Brock 1991; Royle et al. 2012). The majority of benefits that drive the emergence and maintenance of family life are thought to be primarily derived from parental care, which can greatly enhance the direct fitness of offspring and the indirect fitness of parents (Costa 2006; Klug et al. 2012; Wong et al. 2013). By providing care, parents are also able to alleviate at least some inherent costs of family life, such as sibling competition (Trivers 1974; Parker 1979; Mock and Parker 1997). However, recent theoretical considerations emphasized the occurrence of additional social processes that can provide key benefits to family members, such as sibling cooperation, thereby promoting the evolution of family life (Kramer and Meunier 2019).

Indeed, recent years have seen increasing attention toward the potential importance of the occurrence and beneficial effects of sibling cooperation (defined as an altruistic [–/+] or mutually beneficial [+/+] behavior that is selected for because of its beneficial effect on the recipient; West et al. 2007b). Sibling cooperation is promoted by kinship (Hamilton 1964a,b; West et al. 2007a) and is traditionally assumed to be associated with obligate and derived social systems. Examples include food sharing in barn owls (*Tyto alba*; Marti 1989), coordinated fledging in house wrens (*Troglodytes aedon*; Bowers et al. 2013), coordinated begging in banded mongooses (*Mungo mungo*; Bell 2007), and social immunity behaviors in eusocial insects (Cremer et al. 2007;

Stow et al. 2007; Hamilton et al. 2011; He et al. 2018). However, the consideration of sibling cooperation as a driver of early social evolution stems from discoveries of this phenomenon in less derived, subsocial systems. Sibling food sharing, for example, is known to occur in families of huntsmen spiders (*Delena cancerides*; Yip and Rayor 2013), black lace-weavers (*Amaurobius ferox*; Kim et al. 2005), and European earwigs (*Forficula auricularia*). In earwigs, sibling cooperation was even shown to be plastically adjusted and increased when parental care was poor (Falk et al. 2014; Kramer et al. 2015). Indeed, it has been suggested that the degree and nature of cooperative sibling interactions may depend on the degree of offspring dependence on parental care: more independent juveniles may benefit from increasing levels of cooperation, whereas offspring unable to survive on their own should in turn compete over limited parental resources, fostering rivalry over cooperation. In a scenario where obligate family life gradually arises from an independent state, sibling cooperation during early, facultative stages of family life evolution may select for delayed dispersal and gregarious behaviors, setting the stage for the continued association of family members and thus possible avenues toward prolonged forms of family life (Kramer and Meunier 2019). Moreover, a recent study suggests that even if facultative care evolves secondarily from an obligate state, sibling cooperation should increase in the more independent offspring, as its benefits can compensate for the reduced level of parental care (Rebar et al. 2020). However, despite the implied importance of sibling interactions in shaping the evolution of family life, whether and to what degree sibling cooperation depends on the level of offspring dependence remains largely unclear. Investigating closely related species exhibiting family life and sharing key life-history strategies but varying in offspring dependency may reveal key differences in cooperative behaviors and help to better understand if and how these behaviors are tied to progressively obligate forms of family life.

Here, we took advantage of a convenient model system: burying beetles of the genus *Nicrophorus* express complex forms of pre- and posthatching biparental care but show extreme differences in offspring dependency on posthatching care (Trumbo 1992; Capodeanu-Nägler et al. 2016). Burying beetles reproduce on small vertebrate carcasses where they form temporary family units usually consisting of a male and a female parent and a varying number of offspring (Pukowski 1933; Eggert and Müller 1997). Prehatching care is mainly composed of the parents converting carrion into an edible nursery by burying a carcass, removing fur or feathers, treating the carcass with antimicrobial secretions (Hoback et al. 2004; Cotter and Kilner 2010; Vogel et al. 2017), and chewing a hole into the carcass (hereafter referred to as feeding cavity) to facilitate larval access to the resource (Pukowski 1933; Eggert et al. 1998). In the posthatching phase, the parents guard the carcass and offspring against predators, competitors, and intruding conspecifics while actively feeding the larvae with regurgitated carrion (Pukowski 1933, 1934; Milne and Milne 1976). Larvae also independently feed on the carrion, but there are large differences among species in offspring survival in the absence of posthatching care (Trumbo 1992; Capodeanu-Nägler et al. 2016). In *N. orbicollis*, for example, offspring rarely survive in the absence of parents, whereas in *N. pustulatus*, larval survival does not depend on parental presence. Conversely, offspring dependency in *N. vespilloides* is intermediate, as larval survival and growth in the absence of posthatching care are higher than in *N. orbicollis* but lower than in *N. pustulatus* (Trumbo 1992; Capodeanu-Nägler et al. 2016). The difference in dependency is also reflected in the strength of parent-offspring interactions: in the highly dependent species, larvae beg more, and parents provide them with food more frequently than in the more independent ones (Capodeanu-Nägler et al. 2017).

Hence, if offspring dependency on care determines sibling cooperation, the offspring of the more dependent species might be selected to compete for parental resources and thus not show any sign of cooperation. In contrast, the juveniles of the independent species might show a higher degree of cooperation and the larvae of intermediately dependent species a moderate level of cooperative interactions, complementing the lower benefits of their parents' care.

Cooperation in *Nicrophorus* may thereby take various forms, such as participation in social immunity or production of digestive enzymes (Arce et al. 2013; Ziadie et al. 2019). Indeed, positive correlations between larval density and larval performance have been demonstrated—albeit only in the absence of posthatching care and only when overall density was low (Schrader et al. 2015). However, the occurrence of and mechanisms behind positive effects of larval aggregation and density remain under debate: a study explicitly manipulating the initial brood size could not find any positive effect of larval density on larval mass or survival (Magneville et al. 2018). The authors ascribed the previously observed positive associations between brood size and mean larval mass to shared benign or harsh environmental conditions (e.g., presence/absence of a feeding cavity or microbial load on the carcass) rather than evidence for cooperation (Magneville et al. 2018).

Here, we aimed to shed light on the implications of sibling cooperation in simple social systems on the early evolution of sociality by conducting an experiment using three burying beetle species that vary strongly in offspring dependence on parental care. We tested for the occurrence and degree of cooperation in all three species by manipulating the initial brood size using six different larval densities (six different brood sizes on a standardized carcass). To detect the influence of prehatching care on sibling cooperation, we manipulated the presence of prehatching care by performing experiments under two environmental conditions:

(1) a nonprepared carcass with a standardized feeding cavity and (2) a parentally prepared carcass. Even though nonprepared carcasses received an artificially created opening to serve as a feeding cavity (i.e., providing larvae direct access to food), parental carcass preparation has been shown to mediate further benefits through microbial manipulation, which can, for instance, provide predigestion (Wang and Rozen 2018; Miller et al. 2019) and whose absence was shown to be costly (Shukla et al. 2018a) rendering an unprepared carcass a comparatively harsh environment. Because previous studies detected cooperative behavior only in the absence of posthatching parental care (Schrader et al. 2015), we removed parents in the posthatching phase. Previous studies showed that *N. orbicollis* larvae rarely survive until dispersal without posthatching care (Trumbo 1992; Capodeanu-Nägler et al. 2016), we thus compared the performance of larvae (larval growth and survival) in all three species 24 h after they were assigned to their brood and not at dispersal. This experimental procedure also ensured that the carrion food was not a limited resource. To be able to compare our findings with previous efforts to investigate sibling cooperation (Schrader et al. 2015; Magneville et al. 2018), we additionally measured *N. vespilloides* larval performance at dispersal.

So far, there is no consensus whether sibling cooperation occurs in the intermediately dependent species *N. vespilloides*. Furthermore, the occurrence of sibling cooperation has not yet been investigated in species with far more or less dependence on parental care. Because our experiments are premised on interspecific differences in offspring dependence on parental care, we first of all expected to confirm findings of previous studies, which showed that *N. pustulatus* performed better in the absence of parental care than *N. orbicollis* and *N. vespilloides* (Capodeanu-Nägler et al. 2016, 2018). Second and more importantly, if cooperation depends on the degree of offspring reliance on parental care (Kramer and Meunier 2019), we would expect to see a positive effect of brood size on larval performance, which gradually declines with increasing dependence across the three species. Third, we expected that the larvae grow faster and survive better with increasing brood size in the absence of prehatching care (i.e., on nonprepared carcasses), because harsh environments have been shown to promote mutually beneficial interactions (Falk et al. 2014; Schrader et al. 2015; Kramer and Meunier 2019).

Methods

ORIGIN AND HUSBANDRY OF BEETLES

All beetles used in our experiments are descendants of beetles collected from carrion-baited pitfall traps (pork, beef, and chicken offal). *Nicrophorus orbicollis* were collected near Big Falls, Wisconsin, USA (44°36′59.0″ N, 89°00′58.0″ W); *N. pustulatus* were collected near Lexington, Illinois, USA (40°39′57″ N, 88°53′49″ W); and *N. vespilloides* were collected in Bayreuth, Germany (49°55′15.6″ N, 11°34′19.2″ E). Up to five same-sex siblings were kept in boxes (10 × 10 × 6 cm) two thirds filled with moist peat in a 16:8 L:D cycle at 20°C. Beetles were fed biweekly with cut mealworms (*Tenebrio molitor* and *Zophobas morio*) *ad libitum*. Note that the parents of the larvae we used in these experiments were reared with full parental care for several generations under laboratory conditions (*N. vespilloides* were reared for two and three generations, *N. orbicollis* were reared for nine and 10 generations, and *N. pustulatus* were reared for 10 and 11 generations).

EXPERIMENTAL DESIGN AND PROCEDURES

To test for the occurrence and compare the degree of sibling cooperation in the three species in the absence of posthatching care, in all three species, we manipulated the initial brood size (1, 2, 3, 5, 10, or 15 larvae) under two environmental conditions: broods were either established on nonprepared mouse carcasses with a standardized feeding cavity (no prehatching care) or on parentally prepared mouse carcasses (i.e., those larvae received full prehatching care). To enable direct comparison among species, we standardized conditions choosing a carcass size of approximately 20 g that can be used by all three species successfully (Capodeanu-Nägler et al. 2016) and a maximum brood size (15 larvae) that allow larvae to feed *ad libitum*. To generate larvae and parentally manipulated carcasses, we set up between 130 and 150 pairs per species. To this end, nonsibling virgin males and females were paired haphazardly in small boxes (10 × 10 × 6 cm) filled one third with moist peat. Each pair was provided with a thawed mouse carcass 17.5–22.5 g (mean: 20.42 g ± 1.32 SD; Frostfutter.de—BAF Group GmbH, Germany) and placed in a dark climate chamber at 20°C to breed. Twenty-four hours before larvae were expected to hatch (expected start of hatching: *N. orbicollis*: 96 h; *N. pustulatus*: 120 h, *N. vespilloides*: 60 h), parents and carcass were placed in a new box to prevent contact between parents and hatched larvae. By this time, the female completed oviposition, and larval hatching had not occurred yet. The old boxes containing the eggs were checked several times a day (at least every 8 h) for larval hatching. Within each species, newly hatched larvae were pooled to control for within-family variation and individual differences. Pooled larvae were collected in a Petri dish with moist filter paper, weighed, and haphazardly assigned to the different treatment groups. Note that, in species with no evidence for kin or non-kin recognition among larvae and/or parents, offspring pooling is a common procedure among studies investigating sibling cooperation, for example, in the house wren (*Troglodytes aedon*; Bowers et al. 2013), the European earwig (*Forficula auricularia*; Kramer et al. 2015), and also burying beetles (Smiseth et al. 2003; Rebar et al. 2020). Larval mass was determined using a precision scale (Kern ABJ120-4NM, Kern und Sohn GmbH, Balingen, Germany; accuracy 0.01 mg). We placed one, two, three, five, 10, or 15 larvae on one of two carcass types: either a nonprepared or a parentally prepared carcass. To obtain parentally prepared carcasses, we separated the abovementioned beetle pairs from their carrion resource as soon as their larvae had hatched. Furthermore, similar to the study of Magneville et al. (2018), we cut a small hole in the fresh, nonprepared carcasses and did the same to the prepared carcasses if they lacked a parentally established feeding cavity. Overall, we established 240 broods containing *N. orbicollis* larvae ($n = 20$ per treatment), 240 broods containing *N. pustulatus* larvae ($n = 20$ per treatment), and 228 broods containing *N. vespilloides* larvae ($n = 19$ per treatment). Because previous studies could show that the majority of the highly dependent *N. orbicollis* larvae do not survive in the absence of posthatching care until dispersal (Trumbo 1992; Capodeanu-Nägler et al. 2016; we could confirm these results also in the current study, as only 25 of 1440 larvae survived to dispersal; see Fig. S1 for the survival rate per brood until dispersal for all three species), we measured larval performance at hatching and 24 h after hatching in all species. To this end, we gently removed the larvae from the feeding cavity, counted them, measured their mass, and returned them to their carcass. To be able to compare our findings with previous efforts to investigate sibling cooperation (Schrader et al. 2015; Magneville et al. 2018), we additionally measured larval mass and survival in *N. vespilloides* at the time of dispersal, that is, when the larvae left the remains of the carrion resource for pupation.

STATISTICAL ANALYSES

All data were analyzed and plotted using R version 4.1.0 (R Core Team 2021), loaded with the packages “car 3.0-10”, “broom 0.7.6”, and “Hmisc 4.5-0”. Plots were generated using the packages “cowplot 1.1.1”, “ggplot2 3.3.3”, and “ggnewscale 0.4.5”. Two measures of larval performance were used as dependent variables: mean larval growth rate and survival rate per brood in the first 24 h after larval hatching. The growth rate was calculated using the formula $(\mathrm{lm}_{24} - \mathrm{lm}_0)/\mathrm{lm}_0$, where lm_{24} is the mean larval mass of the brood at 24 h and lm_0 the average larval mass of the brood at hatching. We used growth rate instead of larval mass as a measure of offspring performance because species show differences in egg size and therefore in the mass of hatchlings (hatching mass: *N. orbicollis* = 2.87 mg ± 0.30 SD ($n = 240$); *N. pustulatus* = 1.70 mg ± 0.18 SD ($n = 240$), *N. vespilloides* = 2.83 mg ± 0.29 SD ($n = 228$); GLM with Gaussian error: $F_{2,705} = 1543.80$, $P < 0.001$). Hence, for species comparison, growth rate is a more meaningful measurement of larval performance than the absolute mass. To test for effects on larval growth rate, we used generalized linear models (GLM) with Gaussian errors and a logit link function. We applied GLMs with a quasibinomial distribution and a logit link function to test for effects on larval survival rate per brood. We first performed both types of GLMs with species (*N. orbicollis*, *N. pustulatus*, and *N. vespilloides*), carcass type (nonprepared or parentally prepared mouse carcasses), and brood size (as a continuous variable), and all possible interactions of those variables as fixed factors. We obtained *P*-values for the general effects using the “Anova” function with type “III” sum of squares (Sum Sq) of the “car” package. Because we found significant two-way interactions between carcass type and species and carcass type and brood size, we split the dataset and ran separate GLMs for each species and each carcass type to determine how the interactions arose.

Furthermore, to be able to compare our data on *N. vespilloides* with the results of Magneville et al. (2018) and Schrader et al. (2015), we (1) tested for effects of initial brood size and carcass type on mean larval mass and survival rate at the time of dispersal and (2) analyzed the effects of the number of dispersing offspring (brood size at dispersal) on mean larval mass. When mean larval mass was the dependent variable, we used a GLM with Gaussian error and a logit link function; when survival rate was the dependent variable, we performed GLMs with a quasibinomial distribution and a logit link function. As we were particularly interested in whether larvae performed differently on each carcass type, we split the dataset and ran separate GLMs for each carcass type. Here, we used the “summary” function to obtain the estimates and *t*-values for the effect of initial brood size on larval performance.

Results

LARVAL DEPENDENCY PER SPECIES

As predicted and confirming the findings of previous studies on offspring dependency, the larvae of the three species differed significantly in both growth rate (Table 1) and survival rate per brood (Table 1). The larvae of the most independent species (*N. pustulatus*) showed the highest, and the larvae of the most dependent species (*N. orbicollis*) the lowest growth and survival rate in the absence of parents (Figs. 1A and 2A). The larvae of the intermediately dependent species *N. vespilloides* showed a corresponding intermediary growth and survival rate.

EFFECTS OF SPECIES, BROOD SIZE, AND CARCASS TYPE ON LARVAL PERFORMANCE

We would have expected a positive effect of brood size on larval performance (i.e., growth and survival rate), which (1) gradually declines with increasing dependence across the three species and (2) intensifies in the absence of prehatching care. We indeed found an effect of brood size on larval growth and survival rate that was dependent on carcass type (Table 1). Although on nonprepared carcasses, larval growth and survival rate increased with

Table 1. Results of generalized linear models (fitted with a Gaussian error distribution for growth rate and quasibinomial distribution for survival rate, and a logit link function each) examining the effects of species (*N. orbicollis*, *N. pustulatus*, or *N. vespilloides*), carcass type (nonprepared or parentally prepared), initial brood size (as a continuous variable), and their interactions on larval growth and survival rate in the first 24 h after hatching. Larval growth and survival rate were analyzed separately. Significant values are in bold.

	Growth rate				Survival rate			
Predictors	Sum Sq	df	*F*	*P*-value	Sum Sq	df	*F*	*P*-value
Species	16.56	2	32.05	**<0.001**	56.93	2	52.67	**<0.001**
Carcass type	1.74	1	6.75	**0.0096**	0.02	1	0.033	0.86
Brood size	0.67	1	2.58	0.11	6.67	1	12.35	**<0.001**
Species × Carcass type	3.95	2	7.64	**<0.001**	2.49	2	2.31	0.10
Species × Brood size	0.23	2	0.44	0.64	1.08	2	1.00	0.37
Carcass type × Brood size	1.00	1	3.88	**0.049**	2.12	1	3.93	**0.048**
Species × Carcass type × Brood size	0.93	2	1.80	0.17	0.11	2	0.10	0.90
Residuals	128.86	499			376.17	696		

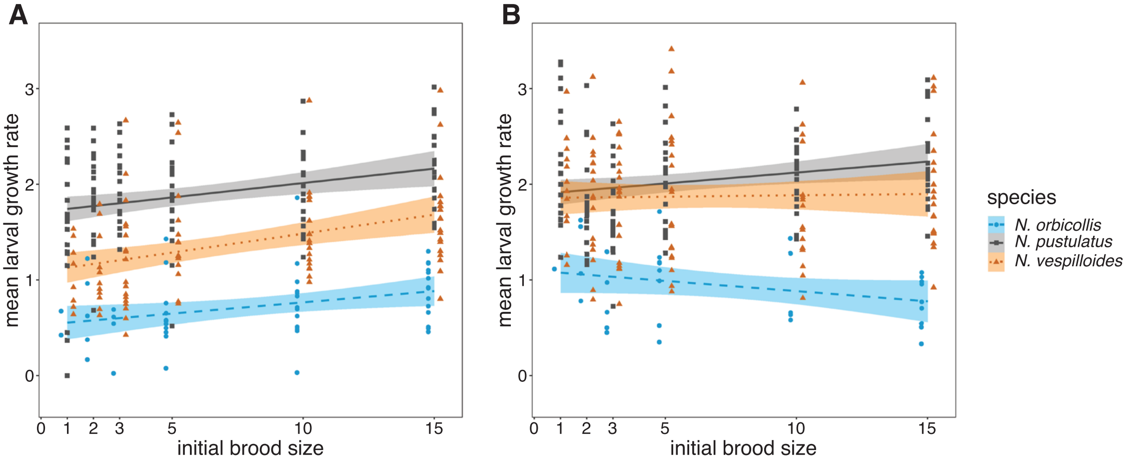

Figure 1. The relationship between initial brood size and mean larval growth rate per brood in the first 24 h after hatching on (A) nonprepared carcasses and (B) parentally prepared carcasses in the three burying beetle species *N. orbicollis* (*n* = 84), *N. pustulatus* (*n* = 234), and *N. vespilloides* (*n* = 194). Each data point represents one brood; the lines are smoothed regression lines and the shaded regions their 95% confidence intervals.

an increase of brood size, larval growth and survival were not affected by brood size on prepared carcasses (Fig. 1 and Fig. 2). Contrary to our expectation, we neither found a two-way interaction between brood size and species nor a three-way interaction between brood size, carcass type, and species (Table 1). However, graphical inspection, as well as separate GLMs for each species and carcass type, revealed a more complex picture. In all three species, larval growth rate increased with brood size on nonprepared carcasses (Fig. 1A; Table 2). On carcasses that had been prepared by parents, only *N. pustulatus* larvae grew better with increasing brood size, whereas in *N. orbicollis* and *N. vespilloides*, brood size had no effect on larval growth rate (Fig. 1B; Table 2). Consequently, the detected interaction effect between brood size and carcass type on larval growth rate (Table 1) was driven by *N. orbicollis* and *N. vespilloides* larvae. With respect to survival rate, we found no effect of brood size when larvae were placed on parentally prepared carcasses in all three species (Fig. 2B; Table 3). On nonprepared carcasses, we detected a positive effect of brood size on larval survival rate in *N. orbicollis* and *N. vespilloides* (Fig. 2A; Table 3). In *N. pustulatus*, no such effect could be revealed (Fig. 2A; Table 3). Hence, also regarding the survival rate, the detected interaction effect between brood size and carcass type (Table 1) was driven by *N. orbicollis* and *N. vespilloides* larvae. However, we emphasize that

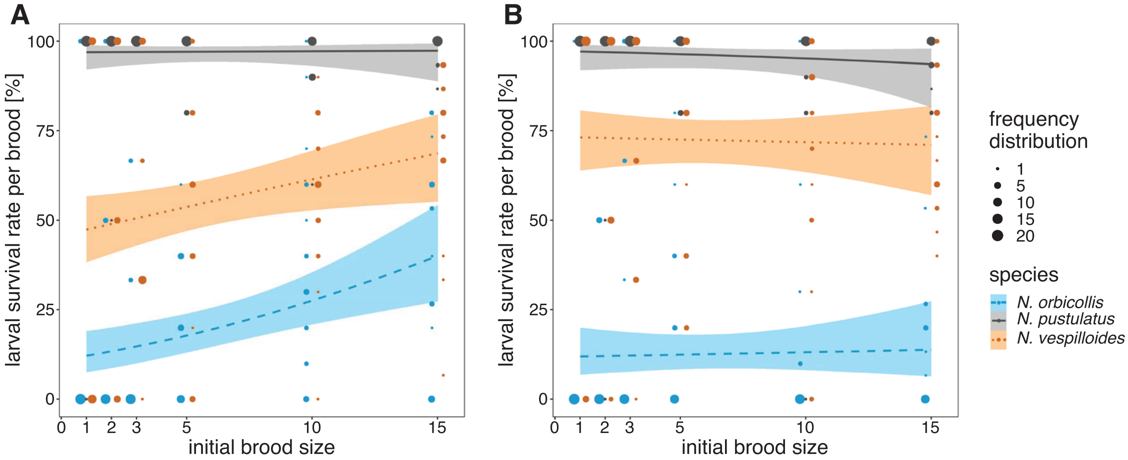

Figure 2. The relationship between initial brood size and larval survival rate per brood in the first 24 h after hatching on (A) nonprepared carcasses and (B) parentally prepared carcasses in the three burying beetle species *N. orbicollis* (*n* = 240), *N. pustulatus* (*n* = 240), and *N. vespilloides* (*n* = 228). The sizes of data points represent the number of broods with identical survival rates per brood. The lines are smoothed regression lines and the shaded regions represent their 95% confidence intervals.

Table 2. Results of generalized linear models (fitted with a Gaussian error distribution and a logit link function) examining the effect of initial brood size (as a continuous variable) on larval growth rate per brood in the first 24 h after hatching in the three species *N. orbicollis*, *N. pustulatus*, and *N. vespilloides*. Larvae either developed on non-prepared or parentally prepared carcasses. The denominator degrees of freedom are indicated by df_d, whereas the numerator degrees of freedom were 1 in all cases. Each combination of carcass type and species was analyzed separately. Significant values are in bold.

		Nonprepared carcass					Prepared carcass				
Species	Terms	Estimate	SE	df_d	*t*-value	*P*-value	Estimate	SE	df_d	*t*-value	*P*-value
N. orbicollis	Intercept	0.53	0.10	51	5.46	<0.001	1.10	0.12	31	9.38	<0.001
	Brood size	0.023	0.0099	50	2.38	**0.021**	–0.021	0.013	30	–1.70	0.099
N. pustulatus	Intercept	1.71	0.072	117	23.72	<0.001	1.90	0.074	115	25.66	<0.001
	Brood size	0.030	0.0092	116	3.27	**0.0014**	0.023	0.0093	114	2.45	**0.016**
N. vespilloides	Intercept	1.09	0.090	89	12.41	<0.001	1.90	0.10	102	18.83	<0.001
	Brood size	0.040	0.010	88	3.87	**<0.001**	–0.000069	0.0124	101	–0.056	0.96

Table 3. Results of generalized linear models (fitted with a quasibinomial error distribution and a logit link function) examining the effect of initial brood size (as a continuous variable) on larval survival rate in the first 24 h after hatching in the species *N. orbicollis*, *N. pustulatus*, and *N. vespilloides*. Larvae either developed on nonprepared or parentally prepared carcasses. The denominator degrees of freedom are indicated by df_d, whereas the numerator degrees of freedom were 1 in all cases. Each combination of carcass type and species was analyzed separately. Significant values are in bold.

		Nonprepared carcass					Prepared carcass				
Species	Terms	Estimate	SE	df_d	*t*-value	*P*-value	Estimate	SE	df_d	*t*-value	*P*-value
N. orbicollis	Intercept	–2.09	0.30	119	–7.06	<0.001	–2.01	0.35	119	–5.81	<0.001
	Brood size	0.11	0.032	118	3.50	**<0.001**	0.012	0.043	118	0.28	0.78
N. pustulatus	Intercept	3.46	0.57	119	6.11	<0.001	3.59	0.61	119	5.86	<0.001
	Brood size	0.0098	0.075	118	0.13	0.90	–0.060	0.067	118	–0.90	0.37
N. vespilloides	Intercept	–0.17	0.21	113	–0.79	0.43	1.01	0.24	113	4.18	<0.001
	Brood size	0.063	0.028	112	2.25	**0.026**	–0.0074	0.031	112	–0.24	0.81

Table 4. Results of generalized linear models (fitted with a Gaussian error distribution for mean larval mass and quasibinomial distribution for survival rate and a logit link function each) examining the effects of carcass type (nonprepared or parentally prepared), initial brood size (as a continuous variable), and their interaction on mean mass and survival rate of *N. vespilloides* larvae until dispersal. Mean larval mass and larval survival rate were analyzed separately. Significant values are in bold.

	Mean larval mass				Larval survival rate			
Predictors	Sum Sq	df	*F*	*P*-value	Sum Sq	df	*F*	*P*-value
Brood size	3286	1	7.49	**0.0071**	8.45	1	17.90	**<0.001**
Carcass type	168	1	0.38	0.54	22.92	1	48.58	**<0.001**
Brood size × Carcass type	774	1	1.76	0.19	2.43	1	5.15	**0.024**
Residuals	58,823	134			106.16	225		

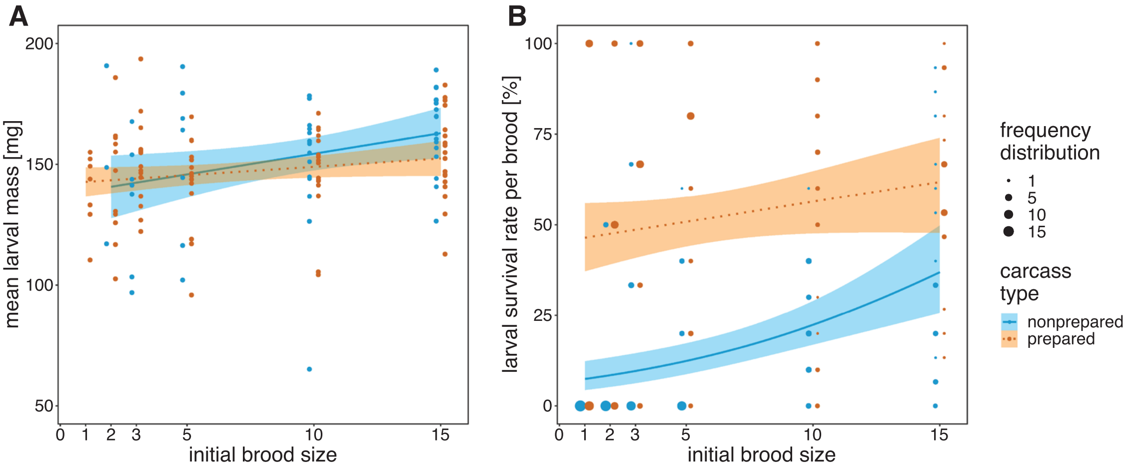

Figure 3. The relationship between initial brood size and two measures of larval performance in *N. vespilloides*: (A) the mean larval mass per brood at dispersal ($n = 138$) and (B) the larval survival rate per brood until dispersal ($n = 228$). Larvae either developed on nonprepared carcasses or parentally prepared carcasses. The sizes of data points represent the number of broods with identical survival per brood in panel (B). The lines are smoothed regression lines and the shaded regions represent their 95% confidence intervals.

in *N. pustulatus*, it was not possible to detect any effect because nearly all larvae survived under both conditions (Fig. 2).

EFFECTS OF BROOD SIZE AND CARCASS TYPE ON *N. vespilloides* PERFORMANCE AFTER DISPERSAL

Focusing on the performance of *N. vespilloides* larvae at the time of dispersal, we found that larval mass was affected by brood size but not by carcass type (Table 4). However, separated GLMs for each carcass type revealed that this effect was driven by larvae on nonprepared carcasses because *N. vespilloides* larvae only gained more weight with an increase of brood size on nonprepared carcasses (Fig. 3A; Table 5).

Similarly, the survival rate until dispersal was also affected by brood size. However, here the effect depended on carcass type (Fig. 3B; Table 4): separated GLMs for each carcass type revealed that only on nonprepared carcasses larval survival increased with an increase in brood size (Table 5).

Finally, we examined the effect of the number of dispersing offspring on larval mass at dispersal (Fig. S2). Separated GLMs for each carcass type revealed that the number of dispersed offspring affected larval mass positively on both carcass types (Table S1).

Discussion

Recent studies have highlighted the importance of cooperative sibling interactions to the emergence and maintenance of family life. In this presumably early, nonderived form of sociality, offspring are not always obligately dependent on their parents, and the degree of offspring cooperative behaviors has been suggested to depend on the degree to which they rely on their parents to grow and survive (Kramer and Meunier 2019). Here, we tested this hypothesis by investigating three different species of the genus *Nicrophorus*, which exhibit dramatically different degrees of dependency on parental care.

Table 5. **Results of generalized linear models (fitted with a Gaussian error distribution for mean larval mass and quasibinomial distribution for survival rate and a logit link function each) examining the effect of initial brood size (as a continuous variable) on mean larval mass at dispersal and larval survival rate from hatching to dispersal in the species *N. vespilloides*. Larvae either developed on nonprepared or parentally prepared carcasses. The denominator degrees of freedom are indicated by df_d, whereas the numerator degrees of freedom were 1 in all cases. Mean larval mass and larval survival rate were analyzed separately for each carcass type. Significant values are in bold.**

		Nonprepared carcass					Prepared carcass				
	Terms	Estimate	SE	df_d	*t*-value	*P*-value	Estimate	SE	df_d	*t*-value	*P*-value
Larval survival rate	Intercept	–2.66	0.31	114	–8.51	<0.001	–0.19	0.21	113	–0.87	0.39
	Brood size	0.14	0.032	113	4.48	**<0.001**	0.044	0.028	112	1.58	0.12
Mean larval mass	Intercept	137.27	7.89	49	17.40	<0.001	142.02	3.37	87	42.15	<0.001
	Brood size	1.71	0.74	48	2.31	**0.025**	0.69	0.39	86	1.78	0.078

We found evidence for sibling cooperation—that is, a positive relationship between brood size and larval growth and survival rate—but its level did not exclusively depend on offspring reliance on parental care. Specifically, we showed that (1) only under harsh environmental conditions, that is, in the absence of prehatching care, larval performance (i.e., larval growth and survival rate) increased with larger brood sizes uniformly across species and thus irrespective of their dependence on parental care, whereas (2) in more benign environmental conditions, that is, in the presence of prehatching care, only the larvae of the independent species *N. pustulatus* showed a positive association of brood size with larval performance. Additionally, we could confirm that the species differ in their reliance on parental care, which was a prerequisite for our hypothesis (Trumbo 1992; Capodeanu-Nägler et al. 2016).

In the absence of care, larvae of the independent *N. pustulatus* showed the highest performance, larvae of the highly dependent *N. orbicollis* showed the lowest performance, and larvae of *N. vespilloides* showed a correspondingly intermediary performance. These findings align with previous studies (Trumbo 1992; Capodeanu-Nägler et al. 2016) and thus lend credence to the hypothesis that if cooperation depends on the degree of offspring reliance on parental care (Kramer and Meunier 2019), we should expect a positive effect of brood size on larval performance gradually declining with increasing dependence across the three species. We furthermore expected that the level of larval cooperation is affected by the harshness of the environment. In line with our second expectation, we found an interaction effect of brood size and carcass type on larval performance. However, contrary to our first expectation, we neither found an interaction effect of brood size and species nor a three-way interaction between brood size, carcass type, and species.

Further analyses revealed that the detected interaction effect of brood size and carcass type on larval growth and survival rate was driven by *N. orbicollis* and *N. vespilloides* larvae. On nonprepared carcasses, all three species showed a positive association of brood size with larval growth rate, whereas on parentally prepared carcasses, this effect was only present in *N. pustulatus* larvae. Regarding larval survival, the interaction of brood size and carcass type was also driven by *N. orbicollis* and *N. vespilloides*. On nonprepared carcasses, *N. orbicollis* and *N. vespilloides* showed an increase of larval survival with brood size, but not on prepared carcasses. Furthermore, this effect was absent for *N. pustulatus* on either carcass type. However, we emphasize that in *N. pustulatus*, it was not possible to detect any effect because nearly all larvae survived under both conditions.

We additionally measured the larval performance of *N. vespilloides* larvae at the time of their dispersal. At high larval densities, larvae consume the carcass entirely and usually compete for food at the end of their development on the resource. However, we nevertheless expected larval performance at dispersal to be similar to the performance after 24 h. This is because we focused on larval cooperation in our study and therefore chose, based on the findings of Schrader et al. (2015), a carcass size (20 g) and a maximum brood size (15) so that competition for carrion food was unlikely to occur. Therefore, larval performance should be positively affected by initial brood size, with a stronger effect in the absence of prehatching care. Indeed, we found that larval mass and survival rate of *N. vespilloides* at dispersal were only affected by brood size in the absence of prehatching care, where larvae performed better with an increase of brood size. In summary, all three species benefit from larger brood sizes in terms of growth when there was no prehatching care, whereas only larvae of the less dependent *N. pustulatus* show this effect in the presence of prehatching care. Similarly, larvae of the two more dependent species survive better in larger broods without prehatching care, but not with prehatching care, whereas less dependent larvae display high survival throughout, possibly masking any such effect. The provision of prehatching care improves larval performance (Eggert et al. 1998); thus, removing prehatching care creates harsh environmental conditions for developing larvae. Prehatching care encompasses the removal of fur or feathers and the application of oral and anal secretions. Parental-derived secretions were shown to (1) increase larval mass (Shukla et al. 2018a), (2) shift the carcasses' microbiome toward beneficial microbes (Duarte et al. 2018; Shukla et al. 2018b), (3) contain antimicrobial peptides (Hall et al. 2011), and (4) contain digestive and detoxifying enzymes (Vogel et al. 2017). Previous studies on *N. vespilloides* suggest that harsh conditions facilitate mutually beneficial interactions (Schrader et al. 2015), whereas benign conditions were shown to facilitate competition among larvae (Smiseth et al. 2007). Harsh environmental conditions also facilitate cooperative interactions in other subsocial insects: in the European earwig (*Forficula auricularia*), it was shown that siblings cooperated more when parental care was poor or absent (Falk et al. 2014). Our data indicate that such cooperative effects can occur in more dependent *Nicrophorus* but are masked by or are absent in the presence of prehatching care, suggesting a possible role of sibling cooperation in buffering the costs of poor or absent parental care in these species. Alternatively, these findings could represent evidence for the role of sibling cooperation as a mediator of the evolution of gregarious behavior, as the mutually beneficial aggregation of larvae may have been selected for prior to the advent of family life in (parts of) this genus.

Larval aggregation has evolved several times and was found to be beneficial in other necrophagous insects, such as the carrion-breeding blowfly (*Lucilia sericata*), where benefits were shown to be mediated by thermal gains and exodigestion of carrion (Scanvion et al. 2018; Charabidze et al. 2021). Similar effects were reported in *Necrodes littoralis*, a carrion beetle related to *Nicrophorus*, where larvae experience group-derived thermal gains like decreased development time and lower mortality from aggregating on the carrion surface (Gruszka et al. 2020). Thus, thermal dynamics represent one prospective mechanism driving the benefits of gregarious behaviors and increased brood sizes. In *Nicrophorus* larvae, effects of such sibling-derived thermal benefits might be more pronounced (or indeed present) in the absence of prehatching care (i.e., nonprepared carcasses) if parental carcass preparation induces similar thermal gains, thereby masking larval effects. Although not investigated in *Nicrophorus*, parentally derived biofilms were also shown to generate heat in *Necrodes littoralis* (Matuszewski and Mądra-Bielewicz 2021).

There are additional, nonmutually exclusive mechanisms that may drive aggregation benefits in *Nicrophorus* larvae. For instance, the positive effects of larger broods may derive from shared costs of digestive enzymes. Larger broods should produce an increased amount of enzymes, which, depending on the proportionality of increase, may reduce the costs of producing such enzymes for each individual. Similar to the hypothesized thermal gains above, this effect may be masked or absent if caring parents provide digestive aid during family life. Additionally, it could be shown that *Nicrophorus* larvae are not only passive recipients of food and immunity-related components: recent studies demonstrated that larvae actively participate by secreting antimicrobials (Arce et al. 2013). Intriguingly, offspring immune gene expression was shown to increase in the absence and decrease in the presence of parental care (Ziadie et al. 2019). This expression adjustment thus represents an important example of offspring dynamically changing their investment into mechanisms underlying important group benefits depending on the presence and quality of parental care. However, as the nature of cooperative behavior in burying beetles is still unclear, we recommend that future research investigate the mechanisms driving the mutual benefits. Moreover, it may aid our understanding of sibling interactions if we determine whether the level of larval gregariousness differs between species and environments.

Taking into consideration that the more independent larvae of *N. pustulatus* appear to benefit from sibling cooperation even in the presence of prehatching care, we suggest two mutually exclusive hypotheses to explain how the absence of prehatching care reveals benefits of larval cooperation in our study. The first hypothesis postulates that sibling cooperation might only be triggered or visible in harsh environmental conditions (i.e., in the absence of parental care) in the more dependent species, whereas it is always exhibited in more independent species, perhaps as a result of selective pressures driven by high rates of parental abandonment or mortality. However, it seems highly unlikely that larvae of species such as *N. orbicollis* with total dependence on care would be selected for buffering against the absence of care (given their complete lack of survival without it). Moreover, *N. orbicollis* may benefit less from parental carcass preparation in the absence of posthatching care than more independent species. In contrast to fresh carcasses, prepared carcasses are characterized by a parentally manipulated microbial community, which primarily helps to preserve and conceal the carcass from other necrophagous animals and conspecific competitors (Suzuki 1999; Trumbo and Sikes 2021; Trumbo et al. 2021). However, the decaying processes of the carcass caused by carcass- and soil-borne microbes might act predigestive, facilitating larval self-feeding, especially among highly dependent species (Capodeanu-Nägler et al. 2016). Such effects might explain why in the first 24 h, *N. orbicollis* survived better on nonprepared carcasses than on parentally prepared carcasses.

The second hypothesis postulates that the mechanisms underlying sibling cooperation might be present in all species regardless of environmental conditions, but their effect might be offset in more dependent species by sibling rivalry arising from competition over parentally derived resources. If larvae compensate for poor or absent parental care with gregarious behavior, the presence of care may concordantly reduce not only larval investment into immunity and the digestion of the carcass but also increase larval investment into competition over limited parental resources: previous studies on *N. vespilloides* suggest that low resource availability intensifies competition for parental feeding among larvae especially in small brood sizes when begging for parental provisioning is more effective than self-feeding (Smiseth and Moore 2002; Botterill-James et al. 2017). Importantly, parental prehatching care might elicit competitive behavior (e.g., over the access to predigested food in the feeding cavity), especially among more dependent species such as *N. orbicollis* and *N. vespilloides*. Thus, negative effects of sibling rivalry might be more pronounced in these species and hence could offset the benefits from sibling cooperation. If this hypothesis is true, then larval aggregation and its positive effects (e.g., group-derived thermal gains or accelerated exodigestion of the carcass by larval excretions) might not have evolved as cooperation in the first place but could be maintained as a mutualistic by-product, that is, a behavior that is mutually beneficial but is not selected for because of its beneficial effect on its recipients.

It is important to note that our results partially align with previous studies investigating the benefits of gregarious behaviors in *Nicrophorus*. Magneville et al. (2018), who also found no effect of brood size on either larval survival or larval mass on prepared carcasses, report no such effect on nonprepared carcasses. Similarly, Schrader et al. (2015) reported evidence for sibling cooperation in the presence of prehatching care—however, the prepared carcasses in their study might more closely resemble the nonprepared that we used. Because Schrader et al. (2015) focused on larval ability to penetrate the carcass and on larval influence on carcass decay, they did not provide the larvae with a feeding cavity (natural or artificial). As a result, the transmission of microbes and enzymatic compounds into the carcass' feeding cavity might also be restricted, compared to the prepared carcasses we used. Another potential explanation for the contrasting effects of brood size on larval performances reported by these studies is the smaller sample sizes for small brood sizes and/or different sized carcasses (7–15 g), a factor known to affect larval behavior (Botterill-James et al. 2017). Additionally, Schrader et al. (2015) based their analyses not on initial brood size but on the number of dispersing offspring. Like Magneville et al. (2018) we could confirm the results of Schrader et al. (2015) when we based our analyzes of mean larval mass with the number of dispersing offspring as a predictor instead of initial brood size (see Fig. S2 and Table S1). We found a positive effect of the number of dispersing offspring not only on nonprepared carcasses but also on prepared carcasses. However, the latter result is correlational and might be caused by the effect of shared environmental conditions (e.g., carcass quality) on both larval survival and growth (Magneville et al. 2018).

In conclusion, our results clearly show that burying beetle larvae benefit from developing in higher densities, possibly caused by gregarious behavior on the carcass. These benefits could, for instance, be mediated by shared costs of digestive, social immunity-related, and/or thermal effects. Interestingly, effects of larval cooperation occurred in all species in the absence of prehatching care, but only in the independent species in the presence of parental care. The presence of benefits of gregariousness in larvae of the closely related *Necrodes littorales* indicates that such effects may already be present in a common ancestor of species both with and without parental care, and the benefits of this apparently ancestral trait are likely to persist with the evolution of elaborate care in burying beetles. These effects of sibling cooperation might have facilitated a prolonged association of parents and their offspring and thus might have blazed the trail for the evolution of more elaborate forms of parental care. To our knowledge, this is the first study investigating how sibling cooperation depends on offspring reliance on parental care in a system in which natural variation of offspring dependence occurs among closely related species. We therefore emphasize the need for further investigation into the mechanisms and evolutionary origins of the observed effects to determine whether they represent ancestral traits that have driven the evolution of family life in burying beetles or whether they have been selected for buffering against poor or absent care.

AUTHOR CONTRIBUTIONS

MAP, LZ, and SS designed the experiment. MAP and LZ conducted the experiment. All authors analyzed the data. MAP wrote the first draft of the manuscript. All authors revised it until completion.

ACKNOWLEDGMENTS

We thank J. C. Creighton, A.-K. Eggert, and S. K. Sakaluk for their assistance with beetle collection. We are grateful for discussions of our ideas with the entire Evolutionary Animal Ecology group at the University of Bayreuth. We also want to thank the two anonymous reviewers, and A. G. McAdam for helpful comments on the manuscript. The study was funded by the University of Bayreuth and a grant from the German Research Foundation (DFG) to SS (STE 1874/6-1).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA ARCHIVING

The DOI for our data is https://doi.org/10.5061/dryad.q573n5tjz.

LITERATURE CITED

Arce, A. N., P. T. Smiseth, and D. E. Rozen. 2013. Antimicrobial secretions and social immunity in larval burying beetles, *Nicrophorus vespilloides*. Anim. Behav. 86:741–745.

Bell, M. B. V. 2007. Cooperative begging in banded mongoose pups. Curr. Biol. 17:717–721.

Botterill-James, T., L. Ford, G. M. While, and P. T. Smiseth. 2017. Resource availability, but not polyandry, influences sibling conflict in a burying beetle *Nicrophorus vespilloides*. Behav. Ecol. 28:1093–1100.

Bowers, K. E., S. K. Sakaluk, and C. F. Thompson. 2013. Sibling cooperation influences the age of nest leaving in an altricial bird. Am. Nat. 181:775–786.

Capodeanu-Nägler, A., E. M. Keppner, H. Vogel, M. Ayasse, A.-K. Eggert, S. K. Sakaluk, and S. Steiger. 2016. From facultative to obligatory parental care: interspecific variation in offspring dependency on post-hatching care in burying beetles. Sci. Rep. 6:29323.

Capodeanu-Nägler, A., A.-K. Eggert, H. Vogel, S. K. Sakaluk, and S. Steiger. 2017. Species divergence in offspring begging and parental provisioning is linked to nutritional dependency. Behav. Ecol. 29:42–50.

Capodeanu-Nägler, A., M. A. Prang, S. T. Trumbo, H. Vogel, A.-K. Eggert, S. K. Sakaluk, and S. Steiger. 2018. Offspring dependence on parental care and the role of parental transfer of oral fluids in burying beetles. Front. Zool. 15:1–12.

Charabidze, D., S. Trumbo, A. Grzywacz, J. T. Costa, M. E. Benbow, P. S. Barton, and S. Matuszewski. 2021. Convergence of social strategies in carrion breeding insects. BioScience 71:1028–1037.

Clutton-Brock, T. H. 1991. The evolution of parental care. Princeton Univ. Press, Princeton, NJ.

Costa, J. T. 2006. The other insect societies. Harvard Univ. Press, Cambridge, MA.

Cotter, S. C., and R. M. Kilner. 2010. Personal immunity versus social immunity. Behav. Ecol. 21:663–668.

Cremer, S., S. A. O. Armitage, and P. Schmid-Hempel. 2007. Social immunity. Curr. Biol. 17:R693–R702.

Duarte, A., M. Welch, C. Swannack, J. Wagner, and R. M. Kilner. 2018. Strategies for managing rival bacterial communities: lessons from burying beetles. J. Anim. Ecol. 87:414–427.

Eggert, A.-K. & J. K. Müller 1997. Biparental care and social evolution in burying beetles: lessons from the larder. Pp. 216–236 *in* J. C. Choe and B. J. Crespi, eds. The evolution of social behavior in insects and arachnids. Cambridge Univ. Press, Cambridge, MA.

Eggert, A.-K., M. Reinking, and J. K. Müller. 1998. Parental care improves offspring survival and growth in burying beetles. Anim. Behav. 55:97–107.

Falk, J., J. W. Y. Wong, M. Kölliker, and J. Meunier. 2014. Sibling cooperation in earwig families provides insights into the early evolution of social life. Am. Nat. 183:547–557.

Gruszka, J., M. Krystkowiak-Kowalska, K. Frątczak-Łagiewska, A. Mądra-Bielewicz, D. Charabidze, and S. Matuszewski. 2020. Patterns and mechanisms for larval aggregation in carrion beetle *Necrodes littoralis* (Coleoptera: Silphidae). Anim. Behav. 162:1–10.

Hall, C. L., N. K. Wadsworth, D. R. Howard, E. M. Jennings, L. D. Farrell, T. S. Magnuson, and R. J. Smith. 2011. Inhibition of microorganisms on a carrion breeding resource: the antimicrobial peptide activity of burying beetle (Coleoptera: Silphidae) oral and anal secretions. Environ. Entomol. 40:669–678.

Hamilton, C., B. T. Lejeune, and R. B. Rosengaus. 2011. Trophallaxis and prophylaxis: social immunity in the carpenter ant *Camponotus pennsylvanicus*. Biol. Lett. 7:89–92.

Hamilton, W. D. 1964a. The genetical evolution of social behaviour. I. J. Theoret. Biol. 7:1–16.

———. 1964b. The genetical evolution of social behaviour. II. J. Theoret. Biol. 7:17–52. https://doi.org/10.1016/0022-5193(64)90039-6

He, S., P. R. Johnston, B. Kuropka, S. Lokatis, C. Weise, R. Plarre, H.-J. Kunte, and D. P. McMahon. 2018. Termite soldiers contribute to social immunity by synthesizing potent oral secretions. Insect Mol. Biol. 27:564–576.

Hoback, W. W., A. A. Bishop, J. Kroemer, J. Scalzitti, and J. J. Shaffer. 2004. Differences among antimicrobial properties of carrion beetle secretions reflect phylogeny and ecology. J. Chem. Ecol. 30:719–729.

Kim, K. W., B. Krafft, and J. C. Choe. 2005. Cooperative prey capture by young subsocial spiders. Behav. Ecol. Sociobiol. 59:92–100.

Klug, H., S. H. Alonzo, and M. B. Bonsall. 2012. Theoretical foundations of parental care. Pp. 21–36 *in* N. J. Royle, P. T. Smiseth, and M. Kölliker, eds. The evolution of parental care. Oxford Univ. Press, Oxford, U.K.

Kramer, J., and J. Meunier. 2019. The other facets of family life and their role in the evolution of animal sociality. Biol. Rev. 94:199–215.

Kramer, J., J. Thesing, and J. Meunier. 2015. Negative association between parental care and sibling cooperation in earwigs: a new perspective on the early evolution of family life? J. Evol. Biol. 28:1299–1308.

Magneville, C., T. Ratz, J. Richardson, and P. T. Smiseth. 2018. No evidence of sibling cooperation in the absence of parental care in *Nicrophorus vespilloides*. Evolution 72:2803–2809.

Marti, C. D. 1989. Food sharing by sibling common barn-owls. Wilson Bull. 101:132–134.

Matuszewski, S., and A. Mądra-Bielewicz. 2021. Heat production in a feeding matrix formed on carrion by communally breeding beetles. Front. Zool. 18:1–11.

Milne, L. J., and M. Milne. 1976. The social behavior of burying beetles. Sci. Am. 235:84–89.

Miller, C. J., S. T. Bates, L. M. Gielda, and J. C. Creighton. 2019. Examining transmission of gut bacteria to preserved carcass via anal secretions in *Nicrophorus defodiens*. PLoS ONE 14:e0225711.

Mock, D. W., and G. A. Parker. 1997. The evolution of sibling rivalry. Oxford Univ. Press, New York.

Parker, G. A. 1979. Sexual selection and sexual conflict. Pp. 123–66 *in* M. S. Blum and N. A. Blum, eds. Sexual selection and reproductive competition in insects. Academic Press, Inc., New York.

Pukowski, E. 1933. Ökologische Untersuchungen an *Necrophorus* F. Z. Morphol. Oekol. Tiere. 27 518–586.

———. 1934. Die Brutpflege des Totengräbers. Entomol. Bl. 30:109–113.

R Core Team. 2021. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/.

Rebar, D., N. W. Bailey, B. J. M. Jarrett, and R. M. Kilner. 2020. An evolutionary switch from sibling rivalry to sibling cooperation, caused by a sustained loss of parental care. Proc. Natl. Acad. Sci. USA 117:2544–2550.

Royle, N. J., P. T. Smiseth, and M. Kölliker. 2012. The evolution of parental care. Oxford Univ. Press, Oxford, U.K.

Scanvion, Q., V. Hédouin, and D. Charabidzé. 2018. Collective exodigestion favours blow fly colonization and development on fresh carcasses. Anim. Behav. 141:221–232.

Schrader, M., B. J. Jarrett, and R. M. Kilner. 2015. Parental care masks a density-dependent shift from cooperation to competition among burying beetle larvae. Evolution 69:1077–1084.

Shukla, S. P., C. Plata, M. Reichelt, S. Steiger, D. G. Heckel, M. Kaltenpoth, A. Vilcinskas, and H. Vogel. 2018a. Microbiome-assisted carrion preservation aids larval development in a burying beetle. Proc. Natl. Acad. Sci. USA 115:11274–11279.

Shukla, S. P., H. Vogel, D. G. Heckel, A. Vilcinskas, and M. Kaltenpoth. 2018b. Burying beetles regulate the microbiome of carcasses and use it to transmit a core microbiota to their offspring. Mol. Ecol. 27:1980–1991.

Smiseth, P. T., and A. J. Moore. 2002. Does resource availability affect offspring begging and parental provisioning in a partially begging species? Anim. Behav. 63:577–585.

Smiseth, P. T., C. T. Darwell, and A. J. Moore. 2003. Partial begging: an empirical model for the early evolution of offspring signalling. Proc. R. Soc. Lond. B Biol. Sci. 270:1773–1777.

Smiseth, P. T., L. Lennox, and A. J. Moore. 2007. Interaction between parental care and sibling competition: parents enhance offspring growth and exacerbate sibling competition. Evolution 61:2331–2339.

Stow, A., D. Briscoe, M. Gillings, M. Holley, S. Smith, R. Leys, T. Silberbauer, C. Turnbull, and A. Beattie. 2007. Antimicrobial defences increase with sociality in bees. Biol. Lett. 3:422–424.

Suzuki, S. 1999. Does carrion-burial by *Nicrophorus vespilloides* (Silphidae: Coleoptera) prevent discovery by other burying beetles? Entomol. Sci. 2:205–208.

Trivers, R. L. 1974. Parent-offspring conflict. Am. Zool. 14:249–264.

Trumbo, S. T. 1992. Monogamy to communal breeding: exploitation of a broad resource base by burying beetles (*Nicrophorus*). Ecol. Entomol. 17:289–298.

Trumbo, S. T., and D. S. Sikes. 2021. Resource concealment and the evolution of parental care in burying beetles. J. Zool. 315:175–182.

Trumbo, S. T., P. K. B. Philbrick, J. Stökl, and S. Steiger. 2021. Burying beetle parents adaptively manipulate information broadcast from a microbial community. Am. Nat. 197:366–378.

Vogel, H., S. P. Shukla, T. Engl, B. Weiss, R. Fischer, S. Steiger, D. G. Heckel, M. Kaltenpoth, and A. Vilcinskas. 2017. The digestive and defensive basis of carcass utilization by the burying beetle and its microbiota. Nat. Commun. 8:1–15.

Wang, Y., and D. E. Rozen. 2018. Gut microbiota in the burying beetle, *Nicrophorus vespilloides*, provide colonization resistance against larval bacterial pathogens. Ecol. Evol. 8:1646–1654.

West, S. A., A. S. Griffin, and A. Gardner. 2007a. Evolutionary explanations for cooperation. Curr. Biol. 17:R661–R672.

———. 2007b. Social semantics: altruism, cooperation, mutualism, strong reciprocity and group selection. J. Evol. Biol. 20 415–432.

Wong, J. W. Y., J. Meunier, and M. Kölliker. 2013. The evolution of parental care in insects: the roles of ecology, life history and the social environment. Ecol. Entomol. 38:123–137.

Yip, E. C., and L. S. Rayor. 2013. The influence of siblings on body condition in a social spider: is prey sharing cooperation or competition? Anim. Behav. 85:1161–1168.

Ziadie, M. A., F. Ebot-Ojong, E. C. McKinney, and A. J. Moore. 2019. Evolution of personal and social immunity in the context of parental care. Am. Nat. 193:296–308.

Associate Editor: A. G. McAdam
Handling Editor: A. G. McAdam

Supporting Information

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

Figure S1. The larval survival rate per brood until dispersal for each of the three burying beetle species: *N. orbicollis* ($n = 240$), *N. pustulatus* ($n = 240$), and *N. vespilloides* ($n = 228$).
Figure S2. The relationship between the number of dispersing offspring and the mean larval mass per brood at dispersal in *N. vespilloides* ($n = 228$).
Table S1. Results of generalized linear models (fitted with a Gaussian error distribution and a logit link function) examining the effect of the number of dispersing offspring (as continuous variable) on mean larval mass in the species *N. vespilloides* at dispersal.