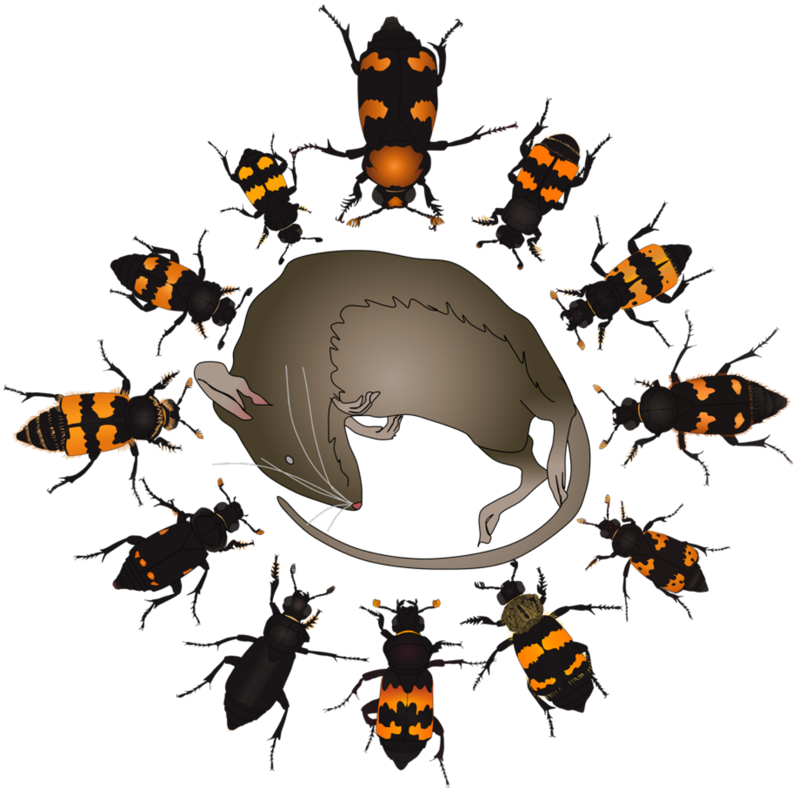
Exploring evolutionary drivers of parental care and family life in burying beetles of the genus *Nicrophorus*



DISSERTATION

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‘It is a wholesome and necessary thing for us to turn again to the earth and in the contemplation of her beauties to know the sense of wonder and humility.’

– ‘The Sense of Wonder’ by **Rachel Carson**, renowned marine biologist, author and conservationist

‘Happiness can be found, even in the darkest of times, if one only remembers to turn on the light.’

– **Professor Albus Percival Wulfric Brian Dumbledore,** Headmaster of Hogwarts School of Witchcraft and Wizardry, a Grand Sorcerer decorated with the Order of Merlin, First Class; Supreme Mugwump of the International Confederation of Wizards and Chief Warlock of the Wizengamot

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Summary

According to current evolutionary theory, the evolution of parental care is a crucial driver for the evolution of social structures and family life. Parental care, which has evolved independently across numerous animal taxa, can take different forms, such as nest site selection and supporting the offspring's food intake. However, environmental conditions and intrafamilial interactions can influence the evolution of care. This dissertation aims to shed light on the complex evolution of parental care and family life in the genus *Nicrophorus*, commonly known as burying beetles. The goal is to clarify the relationship between parental care, offspring dependence, and sibling cooperation. Burying beetles are ideal subjects for studying the evolution of family life because of their remarkable and elaborate parental care behavior. They form temporary family units by reproducing on small vertebrate carcasses, which they bury to supply offspring with food and protection within the carcass nest.

The first part of this dissertation encompasses a common-garden experiment, employing a phylogenetically controlled approach to compare multiple *Nicrophorus* (Coleoptera: Staphylinidae: Silphinae: Nicrophorini) species with related, non-parental Silphini species (Coleoptera: Staphylinidae: Silphinae). Our results demonstrate that parental care in *Nicrophorus* allows offspring to grow faster than their non-parental relatives. We consider this enhanced growth an evolved outcome of parental provisioning and defense mechanisms. Notably, our results highlight differences in growth rate and offspring dependence on parental care across various burying species, which enhances our understanding about the drivers of parental care.

In the second part of this dissertation, I investigated these interspecific differences in offspring dependence on parental care. First, my results confirm the importance of post-hatching care for the survival of the offspring. Second, by examining larval metabolic differences and interspecific variation in larval self-feeding abilities, I revealed the essential role of parental oral secretions in the context of post-hatching care. We found that an easily ingestible paste of carrion alone was not sufficient to ensure larval survival. However, the addition of parental oral secretions to the easily ingested diet of pureed carrion significantly increased the survival of highly dependent *N. orbicollis* larvae, but not to the level of larval survival with parental care. Burying beetles use these oral secretions not only to directly feed their offspring, but also to preserve the vertebrate carcass and form a specific area within the carcass, in which the offspring aggregate, known as the 'feeding cavity'. Because the design of the experiment did not allow us to determine whether these parental-derived oral secretions were intended for direct transfer by parental regurgitation or whether they were intended to modify the feeding cavity, we decided to investigate the parental oral secretions more closely in the third part of this study.

The results of the third part of my dissertation show that both direct and indirect care (food provisioning and carcass modification, respectively) are important, but that direct care, specifically parental regurgitation, has the greatest effect on larval survival. We propose that the oral secretions transferred from parental beetles to their offspring may contain enzymes or growth hormones, similar to what has been observed in some ant species. In the case of burying beetles, existing studies already showed that parental secretions contain beneficial microbes, potentially assisting the larvae in self-feeding from the carcass.

In natural broods of burying beetles, the construction of the feeding cavity is not solely done by the parents. Our second study showed that burying beetle larvae are, to some extent, capable of self-feeding from the carcass. Thus, larvae might be able to manipulate the feeding cavity themselves, thereby increasing their ability to consume food. If this is true, then larvae should develop better if they grow up with siblings (i.e., with increasing brood size) and this effect would be stronger the more independent the offspring are from parental care. We tested these hypotheses and found that larvae benefit from developing with conspecific larvae and suggest that these benefits may be mediated by shared costs associated with digestion, social immunity-related factors, and/or thermal effects. In addition, the social environment was found to play an important role in this context. While all three tested species benefited from developing with conspecific larvae under harsh social environmental conditions (absence of pre-hatching parental care), only the most independent species benefited from developing with conspecific larvae under more favorable conditions, specifically when pre-hatching care was provided.

In summary, this dissertation advances our knowledge of the complex facets of family life in burying beetles. I elucidated certain aspects of the relationships among parental care, offspring dependency, sibling interactions, and social environmental conditions. These findings enhance our understanding of the evolution and mechanisms of parental care and sociality in the animal kingdom.

Zusammenfassung

In der aktuellen Evolutionstheorie wird die Evolution der elterlichen Brutfürsorge als entscheidender Faktor für die Entwicklung sozialer Strukturen und des Familienlebens angesehen. Elterliche Brutfürsorge, die sich in vielen Tiergruppen unabhängig voneinander entwickelt hat, kann verschiedene Formen annehmen, wie z. B. die Wahl des Neststandortes und die Erleichterung der Nahrungsaufnahme durch direkte Fütterung. Jedoch können Umweltbedingungen und innerfamiliäre Interaktionen die Evolution der Brutfürsorge beeinflussen. In dieser Dissertation soll die komplexe Evolution der elterlichen Fürsorge und des Familienlebens in der Gattung *Nicrophorus*, allgemein bekannt als Totengräber, aufgeklärt werden. Ziel ist es, die Beziehung zwischen elterlicher Brutpflege, Abhängigkeit der Nachkommen und Geschwisterkooperation zu klären. Totengräber sind ideale Studienobjekte für die Evolution des Familienlebens aufgrund ihres bemerkenswerten und umfassenden elterlichen Brutpflegeverhaltens. Sie bilden dabei temporäre Familieneinheiten, indem sie kleine Wirbeltierkadaver vergraben und zur Reproduktion nutzen, und so ihren Nachkommen Nahrung und Schutz innerhalb des Kadavernests bieten.

Im ersten Teil dieser Dissertation wurde eine phylogenetisch kontrollierte Studie verwendet, um verschiedene *Nicrophorus*-Arten (Coleoptera: Staphylinidae: Silphinae: Nicrophorini) mit verwandten, nicht elterlichen Silphini-Arten (Coleoptera: Staphylinidae: Silphinae) unter ähnlichen Umweltbedingungen (engl.: *common garden*) zu vergleichen. Die Ergebnisse zeigen, dass die elterliche Brutpflege bei *Nicrophorus* zu einem schnelleren Wachstum der Nachkommen führt als bei ihren nicht-brutfürsorgebetreibenden Verwandten. Dieses gesteigerte Wachstum wird als ein evolutionäres Ergebnis elterlicher Fürsorge gedeutet. Bemerkenswerterweise zeigen die Ergebnisse dieser Studie nicht nur Unterschiede in der Wachstumsrate, sondern auch interspezifische Unterschiede in der Abhängigkeit der Nachkommen von der elterlichen Brutfürsorge innerhalb der Gattung *Nicrophorus* und vertieft so unser Verständnis über die Einflussfaktoren, die auf die elterliche Brutfürsorge wirken.

Im zweiten Teil der Arbeit wurden diese interspezifischen Unterschiede in der Abhängigkeit des Nachwuchses von der elterlichen Brutfürsorge untersucht. Zunächst wurde die Wichtigkeit der postnatalen Brutpflege für das Überleben der Nachkommen bestätigt. Durch die Untersuchung der metabolischen Unterschiede zwischen den Larven und der interspezifischen Variation in der Fähigkeit der Larven, sich selbst zu ernähren, konnte die entscheidende Rolle der elterlichen oralen Sekrete im Zusammenhang mit der postnatalen Brutpflege aufgezeigt werden. Jedoch konnte gezeigt werden, dass eine leicht verdauliche Paste aus püriertem Aas allein nicht ausreicht, um das

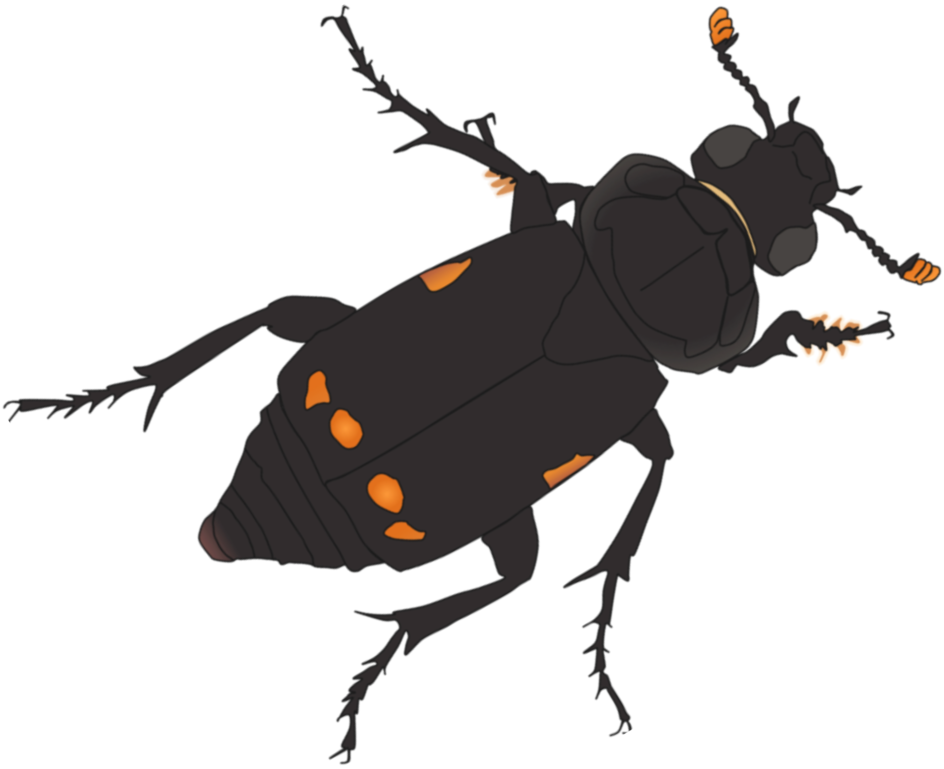
Überleben der Larven zu gewährleisten. Die Zugabe von elterlichen oralen Sekrete zum leicht verdaulichen Futter aus püriertem Aas erhöhte jedoch signifikant das Überleben der stark abhängigen *N. orbicollis*-Larven, jedoch nicht auf das Niveau des Überlebens der Larven mit elterlicher Brutpflege. Totengräber verwenden diese oralen Sekrete nicht nur, um ihren Nachwuchs zu füttern, sondern auch, um den Wirbeltierkadaver zu konservieren und eine spezifische „Futterhöhle“ zu formen, in welcher sich die Nachkommen sammeln. Daher ist auch nicht zu erkennen, ob diese elterlichen oralen Sekrete des Experiments, für die direkte Übertragung durch elterliches Wiederhochwürgen oder für die Modifikation der Futterhöhle bestimmt waren. Im dritten Teil dieser Dissertation wurden daher die elterlichen elterlichen oralen Sekrete genauer untersucht.

In dieser dritten Studie wurde festgestellt, dass sowohl die direkte als auch die indirekte Brutfürsorge (Fütterung bzw. Kadavermodifikation) wichtig sind, dass aber die direkte Brutfürsorge, insbesondere das elterliche Wiederhochwürgen der Nahrung, den größten Einfluss auf das Überleben der Larven hat. Hierbei könnten die elterlichen oralen Sekrete, die von den Elterntieren an ihre Nachkommen weitergegeben werden, Enzyme oder Wachstumshormone enthalten, ähnlich wie dies bei manchen Ameisenarten gezeigt werden konnte. Bei den Totengräbern zeigten frühere Studien bereits, dass die elterlichen oralen Sekrete nützliche Mikroben enthalten, die den Larven möglicherweise bei der Selbstfütterung am Kadaver behilflich sein könnten.

In natürlichen Bruten von Totengräbern wird der Bau der Futterhöhle nicht ausschließlich von den Eltern durchgeführt. Des Weiteren konnte die zweite Studie dieser Dissertation zeigen, dass Totengräberlarven in der Lage sind, sich zumindest teilweise selbständig vom Aas zu ernähren. Daher könnten die Larven auch in der Lage sein, die Futterhöhle selbst zu manipulieren, wodurch ihre Nahrungsaufnahme erhöht werden könnte. Wenn dies der Fall ist, dann sollten die Larven davon profitieren, mit Geschwistern aufzuwachsen (d.h. mit zunehmender Brutgröße) und dieser Effekt sollte umso stärker sein, je unabhängiger der Nachwuchs von der elterlichen Brutpflege ist. Diese Hypothesen wurden in der vierten Studie dieser Dissertation überprüft. Die Ergebnisse zeigen, dass die Larven von der Aufzucht mit Artgenossen profitieren, und dass diese Vorteile möglicherweise durch geteilte Verdauungskosten, Faktoren der sozialen Immunität und/oder thermische Effekte vermittelt werden könnten. Außerdem wurde festgestellt, dass die sozialen Umweltbedingungen in diesem Zusammenhang eine wichtige Rolle spielen. Während alle drei getesteten Arten von der Aufzucht mit Artgenossen unter harten Umweltbedingungen profitierten (ohne pränatale Brutpflege), profitierte nur die unabhängigste Art von der Aufzucht mit Artgenossen unter günstigeren Bedingungen, d.h. wenn pränatale Brutpflege erfolgte.

Zusammenfassend erweitert diese Dissertation unser Wissen über die hochkomplexen Facetten des Familienlebens bei Totengräber. Einige Aspekte der Beziehungen zwischen elterlicher Brutpflege, Abhängigkeit des Nachwuchses, Geschwisterinteraktionen und sozialen Umweltbedingungen konnten aufgedeckt werden, und diese Ergebnisse tragen zu unserem Verständnis der Evolution und der Mechanismen von elterlicher Brutpflege und Sozialität im Tierreich bei.

General Introduction



The evolution of parental care

> ‘For with those animals which were benefited by living in close association, the individuals which took the greatest pleasure in society would best escape various dangers; whilst those that cared least for their comrades and lived solitary would perish in greater numbers. With respect to the origin of the parental and filial affections, which apparently lie at the basis of the social affections, it is hopeless to speculate; but we may infer that they have been to a large extent gained through natural selection.’
>
> – Darwin (1871)

Darwin (1871) first proposed that parental care forms the basis of social behavior, and our understanding of its evolution has expanded rapidly since then (Clutton-Brock 1991; Royle, Smiseth & Kölliker 2012). We now know that the evolution of parental care is a crucial step in the formation of family groups and ultimately in sociality (e.g., Wilson 1975; Szathmáry & Smith 1995; Bourke 2011; Royle, Smiseth & Kölliker 2012; Kramer & Meunier 2019). Although many animal species provide no care for their offspring beyond the small package of yolk that serves as initial nutrition, parental care has evolved independently in a wide range of taxa, including mammals, birds, and invertebrates (e.g., Clutton-Brock 1991; Smiseth, Kölliker & Royle 2012). Parental care is defined by Smiseth, Kölliker and Royle (2012) as ‘any parental trait that enhances the fitness of the parent's offspring and that is likely to have arisen and/or is currently maintained for this function’. Parental care can be provided before or after birth or hatching, and its expression varies enormously across taxa in the animal kingdom and includes but is not limited to behaviors, such as nest building, predator defense and food provisioning (e.g., Clutton-Brock 1991; Costa 2006; Smiseth, Kölliker & Royle 2012).

One of the main functions of parental care is to buffer offspring against abiotic and biotic environmental stress, thereby reducing offspring mortality and significantly increasing both direct offspring fitness and indirect parental fitness (Alonso-Alvarez & Velando 2012; Wong, Meunier & Kölliker 2013; Klug & Bonsall 2014). Harsh environments are thought to be one of the main drivers of sociality. With limited resources and harsh conditions, solitary individuals are likely to face higher mortality rates. However, in this context, the uncertainty of future reproduction also favors parental care, by reducing its relative cost to adults and increasing its potential benefits to offspring (Darwin 1871; Webb *et al.* 2002; Klug & Bonsall 2009; Bonsall & Klug 2011). Costa (2006) summarized the four main environmental factors that drive the evolution of sociality, which can also be applied to the evolution of family life: (i) stable and structured habitats, (ii) high competitive and predation pressure, (iii) unusually stressful physical environments, and (iv) scarce and specialized food sources (see also: Wilson 1975; Krause & Ruxton 2002; Kramer & Meunier 2019). For some species, several of these factors may apply and consequently drive the evolution of family life. For example, in necrophagous insects that use carrion as a breeding resource, such as burying beetles, multiple factors have been hypothesized to play a role; carrion is a scarce, nutritious, and specialized food resource, that is modified by the parents to create a stable and structured habitat for their offspring and to protect their offspring from conspecifics and predators (Pukowski 1933).

Although a substantial body of theoretical and empirical research has been devoted to elucidating the causes underlying the evolutionary origins and maintenance of parental care (Klug, Alonzo & Bonsall 2012), much remains unclear. These investigations have proposed hypotheses that various factors, such as the costs and benefits associated with providing care, specific life-history traits, and ecological and evolutionary dynamics, collectively shape the emergence of parental care (Clutton-Brock 1991; Klug, Alonzo & Bonsall 2012; Royle, Smiseth & Kölliker 2012). Parental care and its evolution are also closely linked to phenomena such as sexual selection, life-history evolution, cooperation and conflict, growth and development, and phenotypic plasticity (Royle, Smiseth & Kölliker 2012).

Although parental care is diverse and widespread in the animal kingdom, not every parental care behavior that promotes offspring survival can be equated with a family. Only when one or both parents are associated with their offspring for an extended period of time after hatching or birth can one speak of family life, which must have additionally emerged and/or be currently maintained to enhance the fitness of the constituent individuals (Kramer & Meunier 2019). In addition, such families are often characterized by intense reciprocal interactions that foster a tightly integrated link between parental care and offspring development (Kramer & Meunier 2019). These interactions contribute to the transfer of resources and facilitate the acquisition of critical life skills, thereby promoting offspring growth and survival. Kramer and Meunier (2019) emphasized in their review that the mechanisms underlying the benefits of parental care and family life may change over evolutionary time, and the factors that once drove the evolution of parental care and family life, may not be the factors that maintain them. To understand the factors that shape the emergence and evolution of parental care, family life, and ultimately sociality, the authors argue, it is crucial, to study not only the derived, and therefore often altricial, evolutionary branches, but also the ancestral states, which are often more precocial (Kramer & Meunier 2019).

Altricial and precocial are the two distinct developmental modes along the continuum of offspring dependence on parental care. Both terms were first described in birds (Baskett 1897), but are also used to refer to mammals and also arthropods (see, e.g., Clutton-Brock 1991; Royle, Smiseth & Kölliker 2012). While precocial species are born relatively mature, and are locomotory active and able to follow their parents soon after hatching, altricial species are often born or hatch underdeveloped, locomotory inactive, and with their eyes closed (Botelho & Faunes 2015); such offspring usually rely on their parents for protection and/or food. Although the traits associated with each developmental mode have been shown to evolve independently across multiple lineages (Botelho & Faunes 2015), parents of altricial and precocial species often exhibit life-history traits and parental care behaviors that algin with the needs of their offspring. For example, parents of precocial species have been shown to often invest in egg size, and clutch size and/or longer gestation or incubation periods compared to altricial species (Rahn, Paganelli & Ar 1975; Martin & MacLarnon 1985; Jetz, Sekercioglu & Böhning-Gaese 2008; Royle, Smiseth & Kölliker 2012; Smiseth, Kölliker & Royle 2012). In contrast, parents in altricial systems typically relax their investment in life-history traits that influence pre-hatching care, such as egg size, incubation, and/or developmental time, because they can compensate for the reduced pre-hatching investment after the offspring hatch or are born (Wesołowski 1994; Williams 1994; Tarwater & Brawn 2010; Royle, Smiseth & Kölliker 2012).

Generally, the expression and maintenance of traits is known to be costly (Lahti *et al.* 2009); however, this also implies that selection for offspring traits that are not used during parental care may be more relaxed. Over time, offspring may evolve traits that facilitate parental care, but that may also hinder offspring development in the absence of parental care (Kramer & Meunier 2019). One such example, is parental food provisioning, the evolution of which is favored when parents are more efficient at feeding offspring than offspring are at self-feeding, or when parents are more efficient at feeding than at guarding against predators (Gardner & Smiseth 2011). When the parents are more efficient at feeding relative to their offspring, the offspring can reduce their investment in traits that promote self-feeding while potentially increasing traits associated with sibling competition. The resulting increase in sibling competition might prompt parents to increase their feeding efforts. These reciprocal relationship reinforces an evolutionary trend in which offspring become increasingly dependent on parental care (Gardner & Smiseth 2011), eventually leading to a parental trap that limits the possibility of parents abandoning their offspring within a species (Eberhard 1975; Wesołowski 1994; Trumbo 2012). However, the evolution and maintenance of offspring dependency is influenced not only by the offspring's social environment, but also by their life-history traits. These traits include egg or clutch size, body size, hatchling or neonate mass, developmental time, growth rate, and potential trade-offs (Wilson 1975; Tallamy & Wood 1986; Bonsall & Klug 2011; Klug, Alonzo & Bonsall 2012; Kramer & Meunier 2019).

To fully understand the complex evolution of family life, it is critical to recognize the importance of all these factors in comparative studies, including species that engage in parental care and species that do not. However, despite the importance of understanding how offspring dependency evolves in many family-living species, there is a lack of comparative studies that experimentally measure offspring dependency. To understand the factors that shape family life and offspring dependency, our best chance is to study species that are closely related but exhibit variations in offspring dependency. The burying beetles (genus *Nicrophorus*), on which I focus in this dissertation, belong to the subfamily of Silphinae within the family of Staphylinidae. The Silphinae subfamily encompasses a wide range of genera and species, exhibiting varying levels of reliance on parental care for their offspring, as well as differences in the complexity and even presence of parental care behaviors. This rich diversity makes it an ideal group for exploring the evolution of family life. Within the Silphinae subfamily, we can distinguish two tribes: the Silphini and the Nicrophorini. The Nicrophorini consists of just one subtribe, known as the Nicrophorina, and these species exhibit varying degrees of parental care. In contrast, the Silphinae subfamily is notable for its lack of any parental care behavior (Ratcliffe 1996; Cai *et al.* 2022; Růžička *et al.* 2023). When comparing differences among species of this diverse subfamily, it is essential to account for taxon-specific variation and thus, the correction for phylogenetic effects is therefore essential to accurately assess offspring dependence and the influence of life-history traits (Freckleton & Harvey 2006).

Life-history traits are closely linked to the developmental process in multicellular organisms, a process that involves growth and differentiation, ultimately leading to increased biomass and the formation of specialized tissues (Roff 1993). From an evolutionary perspective, life-history theory suggests that organisms must optimally allocate energy among the competing demands of growth, maintenance, and reproduction given the specific environmental context (Stearns 1992). In addition, the resource allocation patterns of juveniles can be profoundly influenced by their social environment. In species where juveniles receive parental care, we expect these individuals to allocate fewer resources to maintenance functions, allowing for increased allocation to growth and faster development. In contrast, species with extensive parental care are anticipated to have higher growth rates due to increased resource acquisition through parental regurgitation and reduced investment in self-defense. Such strategies provide benefits to both parents and offspring, contributing to overall fitness (Trivers 1972). To acquire nutrients essential for growth and reproduction, animals consume food, but only a portion of it is absorbed and converted into usable energy or nutrients. This variation in efficiency is influenced by species-specific abilities to process and absorb food, as well as physiological requirements (Castro, Stoyan & Myers 1989; Karasov 1990).

To assist their offspring with food ingestion or digestion, parents in various animal taxa have evolved traits that directly or indirectly provide food for their offspring. For example, mammals directly provide their offspring with milk that is rich in lipids, proteins, minerals, and bioactive compounds, such as growth factors, enzymes and antimicrobial peptides and hormones (Goldman 1993; Ballard & Morrow 2013; Goldman & Chheda 2021). The 'crop milk' of pigeons contains similar compounds, such as pre-digested nutrients, minerals, growth factors, immunoglobulins, and carotenoids (Engberg *et al.* 1992; Shetty *et al.* 1992; Eraud *et al.* 2008). In the genus of wood-feeding cockroaches (*Salganea*), the mothers transfer substances via trophallaxis to their offspring, which have even adapted their mouthparts to facilitate the substance uptake (Nalepa, Bignell & Bandi 2001; Maekawa, Matsumoto & Nalepa 2008; Shimada & Maekawa 2011). Although it remains unclear whether these fluids function to transfer nutrients, microorganisms or enzymes involved in cellulose degradation, it has been shown that the altricial offspring of *S. esakii* suffer high mortality rates when the nymphs are removed from parental care (Nalepa *et al.* 2008). In some cases of parental feeding, parents may pass symbionts to their offspring that are essential for their survival and growth. In another wood-feeding cockroach *Cryptocercus punctulatus,* offspring rely on parental hindgut fluids to establish symbionts necessary for digestion (Cleveland 1934; Nalepa 1990). Burying beetle parents also exhibit similar parental care behaviors. They are able to shape the microbiome on the carcass and introduce symbiotic microbiota including the yeast *Yarrowia,* that is beneficial to the survival of their offspring (Hoback *et al.* 2004; Cotter & Kilner 2010a; Hall *et al.* 2011; Vogel *et al.* 2017; Shukla, Plata, *et al.* 2018; Shukla, Vogel, *et al.* 2018; Miller *et al.* 2019).

Parental care is broadly classified into two categories: direct and indirect. Direct care involves physical contact with the offspring, such as feeding or grooming (Kleiman & Malcolm 1981). In contrast, indirect parental care encompasses behaviors that do not require physical interaction. Examples of indirect care include selecting a suitable oviposition site, nest building and territorial defense (Kleiman & Malcolm 1981). In the case of burying beetles, this includes brood guarding and the inoculation of the carcass with symbiotic microbiota. Although the inoculation may involve physical contact with the offspring, it does not require physical interaction, making it an indirect form of care. While indirect care may seem less valuable than the more elaborate forms of direct care, such as vivipary and food provisioning, both indirect and direct care are important, and in some cases indirect care plays the more critical role in ensuring survival (Clutton-Brock 1991). The extent of indirect parental care can vary greatly within the animal kingdom, ranging from the simple burial of eggs in the substrate to the construction of elaborate nests that provide extensive protection (Smiseth, Kölliker & Royle 2012). For example, nest construction and the selection of a suitable oviposition site provide protection against both biotic and abiotic environmental challenges, shielding offspring from predators as well as adverse conditions such as flooding, desiccation, or extreme temperatures (Smiseth, Kölliker & Royle 2012; Meunier, Körner & Kramer 2022). In some cases, nests even provide additional benefits, such as incorporating antimicrobial plant properties, as in blue tit nests (*Cyanistes caeruleus*; Mennerat *et al.* 2009), or acting as a microbial source and filter (Ruiz-Castellano *et al.* 2016; Campos-Cerda & Bohannan 2020), so that only beneficial bacteria can colonize the nest environment, as in the beewolf *Philanthus triangulum* (Kaltenpoth *et al.* 2005).

Direct and indirect care exhibit immense diversity and complexity in the animal kingdom, but so does the composition, persistence, and intensity of social interactions within family groups generally. Families, as defined earlier, encompasses one or both parents' association with their offspring for an extended period after hatching or birth (Kramer & Meunier 2019). Thus, families may consist of one or more offspring, and may include the mother, father, or both parents. The duration of family groups also varies widely, ranging from a few hours to an entire lifetime (see e.g., Costa 2006). Recent theoretical considerations suggest that, in addition to parental care, there are other social processes (e.g., sibling cooperation and offspring assistance) that provide important benefits to family members, thereby mitigating inherent costs and subsequently promoting the evolution of family life (Kramer, Thesing & Meunier 2015; Kramer & Meunier 2019). For example, sibling cooperation may affect how long families stay together. Sibling cooperation is characterized by altruistic or mutually beneficial behaviors that are selected for because of their positive effects on the recipients (West, Griffin & Gardner 2007b). If offspring benefit from postponing dispersal from their natal/emergence site, the resulting extended periods of family life may subsequently favor family life even before more complex post-hatching parental care evolves (Kramer, Thesing & Meunier 2015; Kramer & Meunier 2019).

Cooperative sibling interactions have also been reported in less derived, subsocial systems, such as huntsman spiders (*Delena cancerides*; Yip & Rayor 2013), black lace-weavers (*Amaurobius ferox*; Kim, Krafft & Choe 2005), and European earwigs (*Forficula auricularia*; Falk *et al.* 2014; Kramer, Thesing & Meunier 2015). Theories suggest that the degree of offspring dependence on parental care influences the extent of cooperative sibling interactions (Kramer & Meunier 2019). However, there are systems, such as burying beetles, in which the effects of offspring aggregation and the classification of sibling interactions as cooperative or competitive remain controversial. Some studies show positive correlations between offspring density and performance, suggesting cooperation, while others attribute these associations to shared environmental conditions rather than true cooperation (Schrader, Jarrett & Kilner 2015a; Magneville *et al.* 2018; Rebar *et al.* 2020; Prang *et al.* 2022). Understanding the complex interactions and cooperative behaviors within families, and recognizing the influence of offspring dependency, provides valuable insights into the evolution, persistence, and ecological significance of family life. By studying the factors that promote or inhibit the spread of family life, we can unravel the complex dynamics that shape social interactions in different animal species.

The study system

Despite the enormous diversity of the order Coleoptera, which stands as the largest order among insects, social behavior remains an uncommon trait in beetles. This makes the members of the genus *Nicrophorus* particularly fascinating, with their remarkably complex family dynamics (Sikes, Madge & Newton 2002; Costa 2006; Sikes, Trumbo & Peck 2016). The 71 species of this genus are widely distributed throughout the northern hemisphere (Pukowski 1933; Bartlett 1987a; Růžička, Háva & Haberer 2000; Sikes & Peck 2000; Sikes, Madge & Trumbo 2006; Sikes & Mousseau 2013).

The reproductive cycle begins when a male or female burying beetle finds a small vertebrate carcass (see **Fig. I**). When the male finds the carcass, he releases a pheromone to attract a female, a process called 'sterzeln' (Pukowski 1933; Eggert & Müller 1989). Once the female arrives, both the male and female may bury the carcass together, or the female may do so alone (Eggert, Reinking & Müller 1998). Both parents have been observed and are generally able to care for their young (Otronen 1988; Robertson 1992; Fetherston, Scott & Traniello 1994; Hocking, Ring & Reimchen 2006), which is unusual for insects. The beetles also often compete for this ephemeral and nutritious resource, and usually the largest species or individual of each sex wins (Wilson & Knollenberg 1984). This often results in a monogamous pair (Pukowski 1933), although large carcasses can sometimes support multiple pairs (Eggert & Müller 1992; Eggert & Sakaluk 2000; for *N. investigator*, where joint breeding is more common see e.g., Hocking, Ring & Reimchen 2006; Hocking *et al.* 2007). Other family constellations, such as single females or single males, are also possible (Wilson & Fudge 1984). The latter, of course, only after the female has finished oviposition. Female behavior in assessing the suitability of the carcass for reproduction, carcass preparation, and burial has been shown to induce ovarian development (Scott & Traniello 1987) and the eggs are laid singly in the soil surrounding the carcass (Pukowski 1933; Engel, Hwang & Steiger 2018).

During pre-hatching care, the carcass is freed from feathers or fur, any fly larvae present are removed, the carcass is rolled into a ball, and covered with antimicrobial peptides from parental oral and anal secretions (Eggert 1992; Eggert & Müller 2000; Rozen, Engelmoer & Smiseth 2008; Cotter & Kilner 2010b; Degenkolb, Düring & Vilcinskas 2011; Steiger *et al.* 2011; Arce *et al.* 2012; Jacobs *et al.* 2016; Vogel *et al.* 2017). By modifying the carcass, the parents preserve the nutritious resource until their larvae hatch. To increase their share of paternity in the brood, males copulate with the female several times during carcass burial (Engel *et al.* 2014), which is necessary because females have almost always mated before and can store sperm for two to three weeks (Eggert

& Müller 2011). Present satellite males or females are deterred from mating with the dominant female or male or from laying their own eggs near the carcass. In the presence of satellites, dominant males increase their copulation attempts (Suzuki 2009) with the dominant female, whereas the dominant female avoids caring for the satellite's larvae by delaying their own oviposition and shortening the larval acceptance window (Scott 1997; Eggert & Müller 2000; Müller *et al.* 2007).

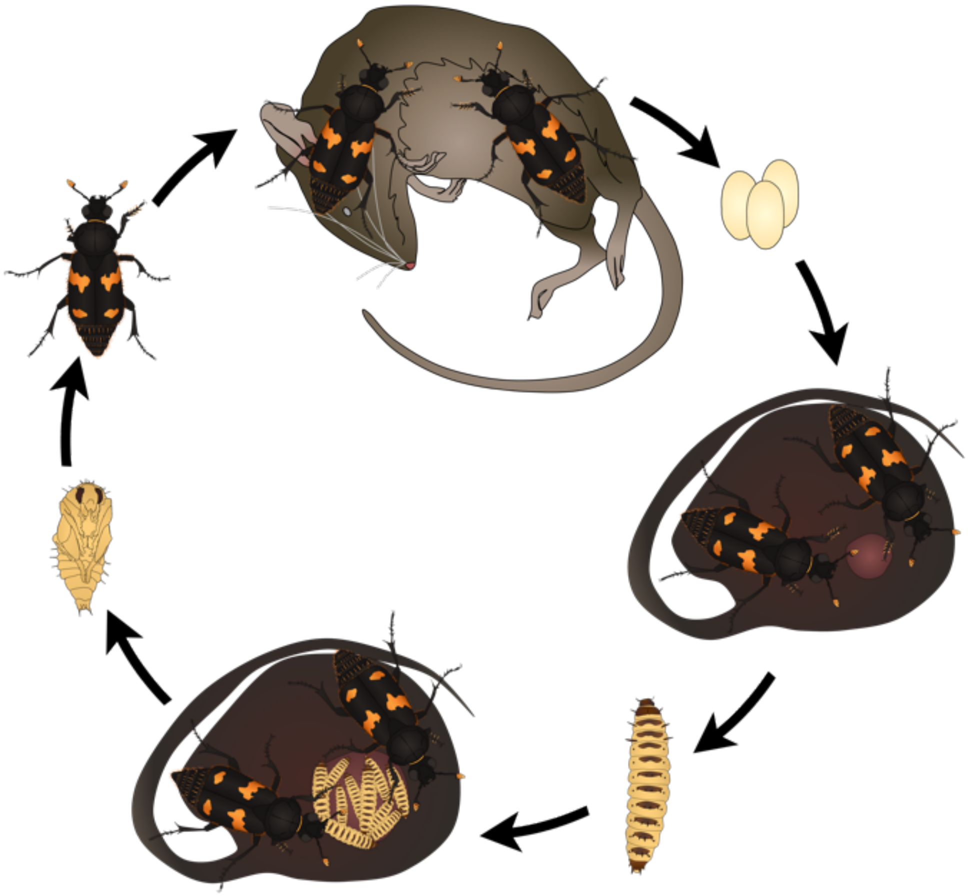


Figure I
Life cycle of burying beetles (*N. orbicollis* as an example)

Parents have two options to adjust their brood size to the carcass size: lay fewer eggs or commit infanticide. Both strategies are used by burying beetles. It has been shown that when *N. vespilloides* is confronted with small carcasses, females can lay fewer eggs in the surrounding soil (Bartlett 1987b; Müller, Eggert & Furlkröger 1990). To have an insurance against hatching failure and egg predation burying beetles generally lay more eggs than the carcass can sustain. This overproduction necessitates infanticide (Bartlett 1987b). Female burying beetles do not recognize their own larvae by chemical cues. Instead, they use a time-dependent cue; females know exactly when their own larvae should hatch and kill anything that arrives at the carcass before then to avoid caring for unrelated larvae (Bartlett 1987b; Müller & Eggert 1990).

Once the larvae hatch, post-hatching care begins, which for most burying beetle species starts around 2-3 days after oviposition, or three to five days after carcass discovery (see **Fig. I**). The hatched larvae are soft and unsclerotized and are guarded and fed by their parents (Pukowski 1933). To prepare the carcass for the arrival of the larvae, the parents facilitate access to the carcass's nutrients by chewing a hole in the integument, creating a feeding cavity (Pukowski 1933). Once the larvae hatch, they crawl onto the carcass and aggregate in the feeding cavity. The feeding cavity benefits the larvae because it allows direct access to the flesh of the carcass and because it contains a biofilm-like matrix of microbes and predigestive enzymes (Shukla, Plata*, et al.* 2018; Shukla, Vogel*, et al.* 2018). While larvae in most species generally have the ability to self-feed, they also engage in begging behavior by touching their parent's mandibles (Rauter & Moore 1999; Smiseth, Darwell & Moore 2003), thereby receiving predigested carrion from their parents.

The carcass is also continuously maintained by the parents (Smiseth, Darwell & Moore 2003). In addition, females produce an anti-aphrodisiac (Engel *et al.* 2016; Engel, Hwang & Steiger 2018), so that both parents focus on caring for the larvae rather than wasting energy on further copulation attempts. During offspring care, parents generally share tasks, however males often spent more time protecting and maintaining the carcass while females spent more time on feeding larvae (Trumbo 2006). However, both parents can adjust flexibly if their partner deserts or dies (Fetherston, Scott & Traniello 1994; Smiseth, Musa & Moore 2006; Creighton *et al.* 2014; Suzuki 2016; Sahm *et al.* 2023). Although burying family units typically last only until the vertebrate carcass is consumed, females remain and provide care until their offspring are nutritionally independent, while the males typically leave earlier (Scott & Traniello 1990). Once the larvae reach their third instar and the carrion is consumed, the larvae are ready to disperse into the surrounding soil to pupate. After the parents leave the carcass, each partner goes its own way, possibly looking for another carcass suitable for a second brood. However, if the first carcass is large enough, sometimes a second brood will develop on the same carcass, and the parents will continue to care for their offspring during this time (Müller 1987; Sahm, Prang & Steiger 2022). Most carrion beetle species pupate approximately one month after the larvae have dispersed, although some species hibernate before completing their pupation (Pukowski 1933; Sikes & Venables 2013; Capodeanu-Nägler *et al.* 2018; see Fig. I).

Furthermore, burying beetle larvae have been shown to differ in their dependence on parental care within the burying beetle genus (Trumbo 1992; Anduaga 2009; Capodeanu-Nägler *et al.*

2016). Offspring dependency generally refers to the extent to which the offspring require parental care to survive. While burying beetle larvae become less dependent on parental care as they age (Trumbo 1992; Eggert, Reinking & Müller 1998), explanations for interspecific differences in offspring dependence among first instar larvae remain unclear.

The study of the evolution of parental care in burying beetles would greatly benefit from a comparative approach that uses large-scale experiments that encompass a wide range of burying beetle species (Coleoptera: Staphylinidae: Silphinae: Nicrophorini: *Nicrophorus*). It should also extend its scope to include closely related species that do not exhibit caregiving behaviors, such as species of the tribe Silphini (Coleoptera: Staphylinidae: Silphinae) while accounting for their phylogenetic relationships, as highlighted by Capodeanu-Nägler *et al.* (2018). Such an approach would provide a comprehensive understanding of the distinctive traits and evolutionary divers of family life in these fascinating carrion beetles.

Aims of this dissertation

Parental care has independently evolved across diverse animal taxa. While parental care can take various forms and is considered one of the main drivers of family life and sociality, there are further processes which influence the evolution of family life. Burying beetles offer an intriguing insight into the complex evolution of parental care and family life, with their remarkable and elaborate parental care. This dissertation aims to advance our knowledge of the complex interplay of factors that influence the evolution of family life in these fascinating beetles, including factors such as parental care, offspring dependency, and social interactions among family members, such as sibling cooperation.

In my first study (**Publication 1: Parental feeding and defence of young facilitate faster offspring growth**), I aim to investigate potential drivers and benefits of the evolution of parental care in burying beetles. Our investigation focuses on species with varying levels of parental care, comparing them to those without such care. We particulary aim to focus on the influence of different life-history traits, such as egg and adult size, on a common trait: offspring growth. To accomplish these goals, we conduct a common garden study that includes species of the tribe Silphini, which do not exhibit parental care and their close relatives, members of the tribe Nicrophorini, which exhibit varying parental care behaviors. Although the tribe Nicrophorini encompasses three genera, we only include the following two: parents of the genus *Nicrophorus* provide both feeding and protection, *Ptomascopus* parents only provide protection. With this comparative approach, we aim to test whether differences in larval growth rates can explain species variation in adult size and offspring dependence among species, as suggested by previous studies (Trumbo 1992; Jarrett *et al.* 2017).

In my second study (**Publication 2: Offspring dependence on parental care and the role of parental transfer of oral fluids in burying beetles**), I focus on the differences in offspring traits and benefits of parental care for three different species within the genus *Nicrophorus*. My aim is to investigate whether variations in these traits account for the observed differences in offspring dependence. First, I want to focus on assessing the offspring's tolerance to starvation. Since all larvae start their development with similar hatching masses, their mortality rates provide insight into their metabolic rates – whether they display a fast or slow metabolism. Faster growth is usually associated with greater food requirements and higher metabolic rates, making fast-growing individuals more susceptible to starvation when resources are limited. However, resource limitation can also occur when an individual struggles to effectively use the provided food resource. To address this, I conduct additional investigations into the larvae's ability to self-feed, using food resources with varying levels of ingestibility. We can even eliminate the potential influence of larval mandibular serration, which was suggested by Benowitz *et al.* (2018) as a possible cause of variations in offspring dependence, by liquefying the carrion food. However, this approach requires considering that parental burying beetles offer their larvae not only liquefied carrion but also supply them with oral secretions that might contain vital enzymes or hormones. To assess the significance of these oral secretions for offspring survival, we introduce small quantities of parental oral secretions to the liquefied carrion food, with a particular focus on securing the survival of the highly dependent burying beetle species *N. orbicollis*. While this helps evaluating the importance of oral secretions, it is important not to overlook the benefits of indirect parental care through the modification of the carcass. To assess the importance of parental care, we determine the duration of care required to ensure larval survival in the highly dependent species *N. orbicollis*. We achieve this by gradually removing parents at specific time intervals during post-hatching care. Combined with the findings from our investigation of the effects of introducing parental oral secretions to liquefied carrion meal, we aim to determine how important specific components of parental care are. These results also prompt my next study to investigate the role of parental oral secretions in both carcass modification and the direct feeding of burying beetle larvae.

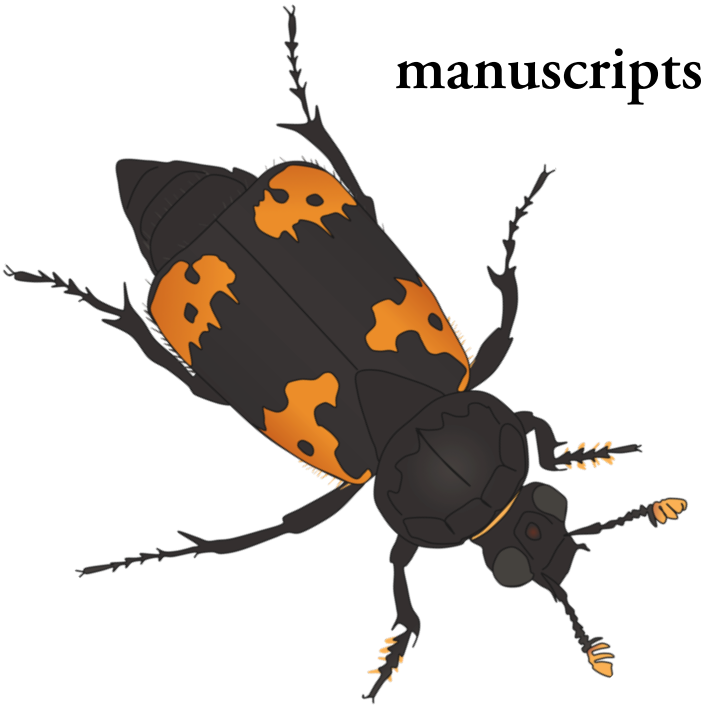
In my third study (**Publication 3: From nutritious nests to parental provisioning: Unveiling the intricate balance of direct and indirect parental care in a highly dependent system**), we explore the benefits and intricate balance between direct and indirect parental care in a highly dependent system, the burying beetle, *N. orbicollis*. As outlined before, parental care enhances the offspring‘s fitness, yet there can occur various forms of parental care within a single system. The carcass modification of burying beetle parents falls under the category of indirect care, as the modification of the carcass does not require interaction between parents and offspring. In contrast, direct care involves physical contact between parents and larvae, notably during parental feeding. Our previous study highlights the importance of parental oral secretions in this context. In this study, we therefore aim to dissect the roles of both direct care (parental feeding) and indirect care (carcass modification) on offspring growth. Additionally, building on the insights from our earlier work, we also investigate the interplay of the duration of care on the effects of direct and indirect care on offspring performance. In summary, we aim to elucidate the intricate dynamics and relationships between direct and indirect parental care, and to examine their benefits on offspring development and survival.

My fourth study (**Publication 4: Differences in sibling cooperation in presence and absence of parental care in a genus with interspecific variation in offspring dependence**) takes a broader perspective on the drivers of parental care in burying beetles by investigating the social dynamics among offspring within the burying beetle family life. Because social dynamics are diverse and complex, I focus on the benefits of sibling interactions. Previous studies suggested that the extent and nature of sibling cooperation depend on the degree of offspring dependence on parental care (Kramer & Meunier 2019). In other words, species with greater independence may benefit more from cooperating with their siblings, while those dependent on parental care might benefit more from competing with their siblings for limited resources. To test this, we first need to establish whether there is sibling cooperation in burying beetles in a comparative framework. This is especially important, because previous studies found contradicting results concerning the occurrence of sibling cooperation in burying beetles (Smiseth, Lennox & Moore 2007; Schrader, Jarrett & Kilner 2015a; Magneville *et al.* 2018). We aim to measure the occurrence and the degree of sibling cooperation by examining the relationship between brood size, larval growth, and survival using three species of the genus *Nicrophorus* with varying degrees of dependence on parental care. However, because the benefits of cooperating with siblings might change in different environmental conditions, we subject the broods to two different environments. The larvae are either placed on a fresh mouse (harsh environment), which lacks the beneficial modification of the parental beetles, or on a parentally prepared carcass (benign environment). This study aims to shed light on the benefits and the evolutionary drivers of family life in these fascinating beetles. Moreover, it might offer conclusions about the existence of sibling cooperation in the ancestors of the *Nicrophorus* genus.

In summary with this dissertation, I try to advance our knowledge of the complex facets of parental care and family life in burying beetles. My goal is to explore the benefits and the evolutionary drivers of parental care and family life and elucidate the relationships of parental care, offspring dependency, sibling interactions, and social environmental conditions. With these findings I aim to enhance our understanding of the evolution and mechanisms of parental care and sociality in the animal kingdom.

Synopsis

A summary of my publications and manuscripts of this dissertation



Publication 1: Parental feeding and defence of young facilitate faster offspring growth

Anne-Katrin Eggert*, **Madlen A. Prang***, Alexandra Capodeanu-Nägler, Mamoru Takata, J. Curtis Creighton, Wenbe Hwang, Scott K. Sakaluk, Derek S. Sikes, Ashlee N. Smith, Seizi Suzuki, Stephen T. Trumbo, and Sandra Steiger

* Anne-Katrin Eggert and **Madlen A. Prang** share first authorship.

Ready to submit

As outlined above (**General Introduction**), many animal species do not provide care for their offspring beyond the small package of yolk that serves as initial nutrition, yet parental care and family life have independently evolved in a wide range of taxa, including mammals, birds, and invertebrates (e.g., Clutton-Brock 1991; Smiseth, Kölliker & Royle 2012). However, in many of these taxa the proximate and ultimate causes for the evolution of parental care remain unclear because the current adaptive value of parental care does not necessarily explain how it initially increased fitness in the ancestral state (see e.g., Williams 1966a). Therefore, to fully understand evolutionary drivers of early family life, it is necessary to consider parental care and its benefits in closely related species which exhibit parental care, and such that do not (Kölliker 2007; Falk *et al.* 2014).

The subfamily Silphinae includes genera and species that vary in the dependence of their offspring on parental care and in the complexity of parental care behaviors, making it ideal for studying the evolution of family life. The subfamily can be divided into the two tribes, the Silphini and the Nicrophorini. The Nicrophorini contain only one subtribe, the Nicrophorina, which show varying degrees of post-hatching parental care. In contrast, the Silphini do not provide care for their offspring (Ratcliffe 1996; Cai *et al.* 2022; Růžička *et al.* 2023).

Because the larvae of the Silphini are not actively protected by their parents, we expect that they must invest more resources in costly anti-predator adaptations such as body armor, defensive secretions (Dorsey 1940; Anderson 1982). In contrast, the parents of the Nicrophorini protect their larvae from predators. Therefore, we expect that their offspring can relax their investment in self-defense and adapt to parental care (Klug & Bonsall 2014). The Nicrophorini includes the genera

Nicrophorus and *Ptomascopus*. Parents of the *Nicrophorus* beetles provide more elaborate post-hatching care compared to *Ptomascopus*; *Nicrophorus* parents do not only protect and defend their offspring, but also conceal them and the food resource (carrion) from predators and competitors, using antimicrobial substances to inhibit carrion nest decay (Pukowski 1933; Pukowski 1934a; Suzuki 1999; Arce *et al.* 2012; Trumbo & Sikes 2021).

To understand potential drivers for the evolution of family life in the burying beetles of the genus *Nicrophorus*, we should use a comparative approach. We should compare traits within the members of the Nicrophorini to understand how they benefit from parental care, but we should also compare the Nicrophorini with closely related species that do not exhibit parental care. We can then compare the influence of potential drivers such as different life-history traits (e.g., egg or body size) on a comparable trait such as offspring growth. Offspring growth rates allow us to infer offspring resource allocation, as any organism must optimally allocate resources (such as time, energy, and nutrients) among the competing demands of growth, maintenance, and reproduction. Parental care may not only protect offspring from various environmental hazards (predators, pathogens, desiccation, flooding, etc.), but may also influence the allocation of resources to offspring growth through provisioning or direct feeding (Wilson 1975; Clutton-Brock 1991; Smiseth, Kölliker & Royle 2012).

In this study (**Publication 1: Parental feeding and defence of young facilitate faster offspring growth**), I first tested how life history traits (e.g., egg size and adult size) influence larval growth rates in species which exhibit parental care and such that do not. I found that offspring growth rates were independent of egg size or the size of larvae dispersing from the carcass, both within the subfamily Silphinae and more specifically also species of the Nicrophorini (see **Fig. 1.1**). This contradicts the suggestion of Trumbo (1992) that larger species achieve their size through faster larval growth.

Since these life history traits do not seem to influence growth rates in Silphinae subfamily, we then tested whether the presence of parental care allows larvae to allocate more resources to their own growth. We showed that early growth rates were highest in *Nicrophorus* (feeding and protection), intermediate in *P. morio* (protection only), and lowest in the tribe Silphini (no parental care), under conditions that mimicked the natural situation (with parents in the tribe Nicrophorini, without in the Silphini; see **Fig. 1.2**). However, when we removed post-hatching parental care in the Nicrophorini, we found intraspecific differences in the growth rate between *Nicrophorus* larvae with and without parental attendance. The lack of faster growth in unattended *Nicrophorus* larvae may be due to limitations in food intake or the absence of traits associated with self-feeding, and these differences in larval growth rates between *Nicrophorus* with or without parental attendance is a direct result of the greater resources assimilated by fed larvae. However, it raises the question of what factors lead to the difference in the ability of burying beetle larvae to assimilate resources in the absence of post-hatching parental care.

Publication 2: Offspring dependence on parental care and the role of parental transfer of oral fluids in burying beetles

Alexandra Capodeanu-Nägler, **Madlen A. Prang**, Stephen T. Trumbo, Heiko Vogel,
Anne-Katrin Eggert, Scott K. Sakaluk, and Sandra Steiger

Published in Frontiers in Zoology 15:33 (29 August 2018)

The first study (**Publication 1: Parental feeding and defense of young facilitate faster offspring growth**) of this dissertation confirmed the intrageneric differences in offspring growth and dependence found in previous studies in the genus *Nicrophorus* (Trumbo 1992; Capodeanu-Nägler *et al.* 2016). Specifically, our study found differences in offspring growth rate in the absence of post-hatching parental care, which are a direct result of differences in larval resource assimilation. However, the question remains why burying beetle larvae differ in their ability to assimilate resources in absence of post-hatching parental care. We attempted to answer this question (**Publication 2: Offspring dependence on parental care and the role of parental transfer of oral fluids in burying beetles**) by comparing three species and focusing on factors that may have contributed to the extreme dependence of *N. orbicollis* offspring on parental care and investigated how these factors vary among three species that differ in offspring dependence.

Our initial test focused on assessing the starvation tolerance of the offspring. Given that the larvae start their development with comparable hatching masses, the mortality rate provides insight into whether these larvae exhibit fast or slow metabolism. Our results show that larval starvation tolerance varies between species (see **Fig. 2.1**). Although the most independent species, *N. pustulatus*, was found to be the most starvation tolerant and to survive the longest in the absence of food, the highly dependent larvae of *N. orbicollis* starved to death later than larvae of *N. vespilloides*, which show an intermediate dependence on parental care. Thus, starvation tolerance alone does not explain differences in dependence on parental care. However, faster growth is usually associated with greater food requirements and higher metabolic rates, making fast-growing individuals such as *N. vespilloides* more susceptible to starvation when resources are limited.

Another study examined the developmental and phylogenetic correlation between offspring independence and larval mandibular morphology and found the presence of serrations on the inner edge of the mandible in independent species (Benowitz *et al.* 2018). Although these findings alone could have explained intrageneric differences in offspring dependence on parental care, the results of our study (**Publication 2: Offspring dependence on parental care and the role of parental transfer of oral fluids in burying beetles**) suggest that this alone cannot explain the differences in offspring dependence. In our experiment, we intentionally eliminated the influence of larval mandibular serration when we liquefied the carrion food. Our results also showed that newly hatched *N. orbicollis* larvae were generally able to self-feed and gain weight when reared on (easy to ingest) baby mice (**see Fig. 2.2** and **Fig. 2.3**), but not when developing on prepared carcasses, which parents typically use as a food resource for their offspring under natural conditions. Our results show that the ability of the highly dependent *N. orbicollis* offspring to use different types of food is more limited than in the more independent species, such as *N. pustulatus* and *N. vespilloides*. In addition, even liquefying the carrion meal was not sufficient to ensure larval survival in the highly dependent *N. orbicollis* (see **Fig. 2.4**).

Based on these results, we decided to focus on the effect of parental modification of the natural food resource. Parental burying beetles not only provide their larvae with a small vertebrate carrion that they modify with beneficial and antimicrobial anal and oral secretions, but they also provide their larvae with oral secretions that are fed directly to the larvae. We found that adding parental oral secretions to the liquefied carrion meal prolonged the survival of *N. orbicollis* larvae when reared in absence of parents, but not long enough to ensure that the larvae pupate (see **Fig. 2.4**). This suggests that *N. orbicollis* larvae require specific enzymes or nutrients that can only be provided by their parents. However, these factors appear to be delivered in sufficient quantities early after hatching, because three hours of post-hatching care was sufficient to significantly increase the survival and final mass of the larvae of the most dependent species, *N. orbicollis*. This raises the question of which components of parental oral secretions are critical for burying beetle larvae. And second, how important are carcass modification and direct feeding, the two parts of parental post-hatching care, in which parental oral secretions are involved.

Publication 3: From nutritious nests to parental provisioning: Unveiling the intricate balance of direct and indirect parental care in a highly dependent system

Madlen A. Prang, Daniela Lauterbach, Patrick Schober, and Sandra Steiger

Ready to submit

As outlined above (**General Introduction**), parental care is defined as 'any parental trait that enhances the fitness of a parent's offspring, and that is likely to have originated and/or to be currently maintained for this function' (Clutton-Brock 1991; Smiseth, Kölliker & Royle 2012). However, this definition does not distinguish between different types of parental care that may occur in the same system. The benefits of indirect care (nest site selection or territory defense) can often be masked by the more elaborate forms of direct parental care (vivipary and food provisioning; see e.g., Kramer & Meunier 2019). This concealment often arises from the frequent co-occurrence of direct and indirect forms of parental care, making it difficult to disentangle their individual effects, especially if offspring are obligately dependent on parental care.

Indirect parental care is generally defined as parental traits that do not require physical contact with the offspring (e.g., the construction of a nest or the selection of a suitable oviposition site; Kleiman & Malcolm 1981). Indirect forms of parental care can vary widely within the animal kingdom, especially nest building, which can range from the simple burial of eggs in the substrate (Baur 1994) to the construction of highly elaborate nests (Winkler & Sheldon 1993; Grubbauer & Hoi 1996). In the context of burying beetles, nest building often involves the modification of food resources and may or may not involve physical contact between parents and offspring. Consequently, we categorize carcass modification as a form of indirect care.

Direct care requires physical contact between parents and larvae, and in burying beetles direct care always occurs when the parents are feeding the larvae, as parental feeding is elicited by parents only after the larva has begged for food by raising its head toward the parent while waving with its legs and touching the parent (Rauter & Moore 1999; Smiseth, Darwell & Moore 2003).

In our previous study (**Publication 2: Offspring dependence on parental care and the role of parental transfer of oral fluids in burying beetles**), we found that *N. orbicollis* larvae rely on parental oral secretions for their survival. We suggest that these parental oral secretions may contain specific enzymes, essential nutrients, and potentially crucial symbionts, as previously detected in parental anal secretions. Parents use oral and anal secretions to modify the carcass, but they only regurgitate their offspring oral secretions containing the pre-digested carrion. Therefore, in this study (**Publication 3: From nutritious nests to parental provisioning: Unveiling the intricate balance of direct and indirect parental care in a highly dependent system**), we investigated the benefits of both direct care (feeding of burying beetle larvae) and the indirect care part of post-hatching parental care (the modification of the carcass) on offspring growth. Additionally, because our previous study (**Publication 2: Offspring dependence on parental care and the role of parental transfer of oral fluids in burying beetles**) found that the essential factors in parental secretions are likely to be transferred in sufficient quantities early - in the first 3 hours after hatching - we also investigated the effect of duration of care on larval performance. Although we found that direct care, such as parental feeding, had a greater effect on promoting larval growth and survival than indirect care (see **Fig. 3.2** and **Fig. 3.3**), both the duration and type of care significantly affected larval performance. Specifically, we found that larvae required at least 12 hours of direct care or 3 hours of a combination of direct and indirect care to survive, while a shorter duration of direct care or only indirect care resulted in higher larval mortality. This study shed light on the benefits of parental oral secretions, providing a clearer understanding of their significance in both direct and indirect parental care.

Publication 4: Differences in sibling cooperation in presence and absence of parental care in a genus with interspecific variation in offspring dependence

Madlen A. Prang, Lena Zywucki, Maximilian Körner, and Sandra Steiger

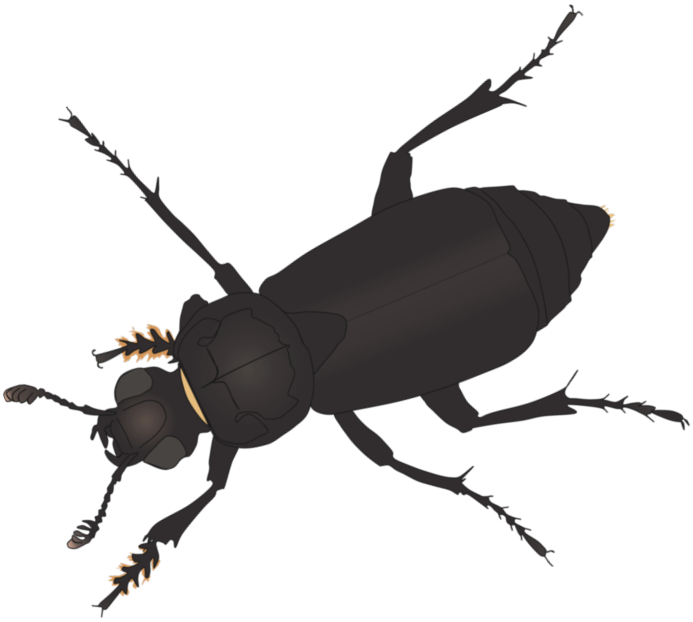
Published in Evolution (01 February 2022)

As outlined above (**General Introduction**), evolutionary drivers and their interactions in the context of parental care and family life, not limited to burying beetles, are highly complex. There are many factors that influence burying beetle family life. Previous studies have demonstrated variations in the growth and mortality of burying beetle offspring in the absence of parental care (Trumbo 1992; Capodeanu-Nägler *et al.* 2016), and it appears that this reliance on parental care is primarily attributed to nutritional dependency (Capodeanu-Nägler *et al.* 2017). Furthermore, interspecific differences in larval begging and parental food provisioning were observed, as well as differences in the morphology of larval mandibles among burying beetle species (Benowitz *et al.* 2018). Recent studies suggest that the social environment of the offspring may have an effect (Kramer & Meunier 2019), but in the context of burying beetles this is highly controversial (Smiseth, Lennox & Moore 2007; Schrader, Jarrett & Kilner 2015a; Magneville *et al.* 2018).

In species where family members live in close proximity, such as burying beetles or earwigs, the effect of sibling interactions may have a greater impact on the evolution of family life than previously expected (Kramer & Meunier 2019). It has been suggested that the extent 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 for limited parental resources, promoting rivalry over cooperation.

Therefore, in this study (**Publication 4: Differences in sibling cooperation in presence and absence of parental care in a genus with interspecific variation in offspring dependence**), we asked whether sibling cooperation is present in burying beetle species and tried to shed light on the question of whether it could have helped to drive the evolution of family life. We focused on 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). For this, I examined the relationship between brood size, larval growth, and survival using three species of the genus *Nicrophorus* with varying degrees of dependence on parental care. We additionally exposed the broods to two different environments, because harsh environmental conditions are known to be a driver of sociality (Costa 2006), and that harsh conditions can alter the nature of offspring interactions. The larvae were either placed on a fresh mouse (harsh environment), which lacks the beneficial modification of the parental beetles, or on a parentally prepared carcass (benign environment).

In this last study (**Publication 4: Differences in sibling cooperation in presence and absence of parental care in a genus with interspecific variation in offspring dependence**), we found evidence for sibling cooperation in burying beetles. Furthermore, we found two factors that influence the level of sibling cooperation. By comparing three species with different levels of offspring dependency, we were able to show that sibling cooperation was influenced by environmental conditions and on offspring dependency on parental care. Larvae of the independent species *N. pustulatus* showed a positive relationship between brood size and larval performance in the presence of pre-hatching care (benign environmental conditions). In contrast, under harsh environmental conditions, larger brood sizes uniformly increased larval performance in all three species (the independent *N. pustulatus*, the intermediary dependent *N. vespilloides* and the dependent *N. orbicollis)*, regardless of their dependence on parental care. These results provide important insights into the transition from facultative to obligate family life and suggests that sibling cooperation may already been present in an early ancestor of the genus *Nicrophorus*.



General Discussion and Perspectives

In this dissertation, I contributed to the knowledge of the evolutionary drivers of family life in burying beetles (genus *Nicrophorus*). I investigated the relationships and benefits of these factors, including parental care, life-history characteristics, offspring dependency, and the dynamics of cooperative sibling interactions. Using a common garden comparative design that included both parental and non-parental carrion beetles (Silphinae; see **Publication 1: Parental feeding and defence of young facilitate faster offspring growth**), I was able to highlight differences in growth rate among parental and non-parental beetles which could not be explained by differences in adult or egg sizes. This enhances our knowledge about potential drivers of parental care in burying beetles and expands our understanding of variation in offspring dependence and physiological and behavioral differences in burying beetle offspring (**Publication 2: Offspring dependence on parental care and the role of parental transfer of oral fluids in burying beetles**). With my third study I elucidate some of the causes of variation in offspring dependence on parental care, and helped to identify one of the specific parental behaviors on which larvae depend (**Publication 3: From nutritious nests to parental provisioning: Unveiling the intricate balance of direct and indirect parental care in a highly dependent system**). I have also investigated the role of the social environment on larval development, with a particular focus on the cooperative interactions of burying beetle larvae. This perspective is crucial for understanding the nuances of family life in its formative stages (**Publication 4: Differences in sibling cooperation in presence and absence of parental care in a genus with interspecific variation in offspring dependence**).

Current evolutionary theory posits that parental care is a major driver of the formation of family groups and subsequently also sociality (Clutton-Brock 1991; Costa 2006; Royle, Smiseth & Kölliker 2012). While many animal species provide no care for their offspring beyond the small package of yolk that serves as initial nutrition, parental care has evolved independently in a variety of animal taxa, including mammals, birds, and invertebrates (Clutton-Brock 1991; Balshine 2012; Royle, Smiseth & Kölliker 2012; Smiseth, Kölliker & Royle 2012). Across this wide spectrum, from species with no parental care to those with highly elaborate care, all share a universal theme: Each organism must optimally allocate energy among the competing demands of growth, maintenance, and reproduction.

To fully understand how an individual allocates its resources, we must consider its specific environmental context (Boggs 2009; Gutiérrez *et al.* 2020), which also includes the individual's social environment. When young individuals experience parental care, the offspring may be shielded by parents against environmental hazards, including predators, pathogens, desiccation, flooding, and more. Furthermore, the parents might enhance or facilitate the offspring’s food intake through provisioning or direct feeding (Wilson 1975; Clutton-Brock 1991; Smiseth, Kölliker & Royle 2012). This leads us to expect, that offspring under such care could reallocate energy from defense to growth and development. As described earlier (**General Introduction**), the reciprocal relationship of parental feeding and the offspring's investment in traits associated with self-feeding reinforces an evolutionary trend in which offspring become increasingly dependent on parental care (Gardner & Smiseth 2011). Interestingly, a previous study revealed that burying beetle species characterized by high levels of offspring dependence were often among the larger species within the *Nicrophorus* genus (Trumbo 1992). This observation led to the suggestion that variances in larval growth rates could explain interspecific differences in adult size and offspring dependence (Trumbo 1992; and echoed by Jarrett *et al.* 2017). If larger species achieve their larger adult size primarily by growing faster, rather than by extending their growth period, we should find a co-variation between adult size and larval growth rate across species. Additionally, as the decision of resource allocation is strongly influenced by an individual's social environment, we would expect that the relationships between life-history traits and growth rate would also be influenced by the evolution of parental care. Moreover, within parental species, these relationships may be further influenced by the presence or absence of parental care.

In my first study (**Publication 1: Parental feeding and defence of young facilitate faster offspring growth**), we used a common garden phylogenetically controlled comparison of several members of the Nicrophorini tribe, which exhibit parental care, and their relatives, the tribe Silphini, which do not exhibit parental care. Here, we found that the evolution of parental care allowed offspring to grow faster than their related nonparental relatives. We considered this faster growth to be an evolved result of parental provisioning, which allowed developing larvae to allocate more resources to their own rapid growth, and parental defense of young, which emancipated *Nicrophorus* larvae from the need to invest in their own costly antipredator adaptations.

Our results showed differences in growth rates that could not be attributed to variations in adult body size or egg size. But we also found interspecific variations in offspring dependence on parental care within the *Nicrophorus* genus (see **Fig. II**). While knowledge about differences in offspring dependency on parental care in burying beetles has been available for over three decades (Trumbo 1992), this intraspecific variation had yet to be investigated using a standardized, comparative approach. Prior studies had relied on data gathered from preexisting yet non-comparable sources, as exemplified by Jarrett *et al.* (2017). Previous studies have attempted to identify the proximate causes of the observed interspecific differences in offspring dependency. In particular, Eggert, Reinking and Müller (1998) highlighted the importance of post-hatching care, a finding that was later confirmed by Capodeanu-Nägler *et al.* (2016). In this study Capodeanu-Nägler *et al.* (2016) focused on three burying beetle species with varying degrees of offspring dependence: the independent *N. pustulatus*, the intermediary dependent *N. vespilloides*, and the highly dependent *N. orbicollis*. Capodeanu-Nägler *et al.* (2016) were also able to show that the variation in offspring dependency found in their own and previous studies is due to differences in dependence on post-hatching care, rather than pre-hatching care (Trumbo 1992; Capodeanu-Nägler *et al.* 2016).

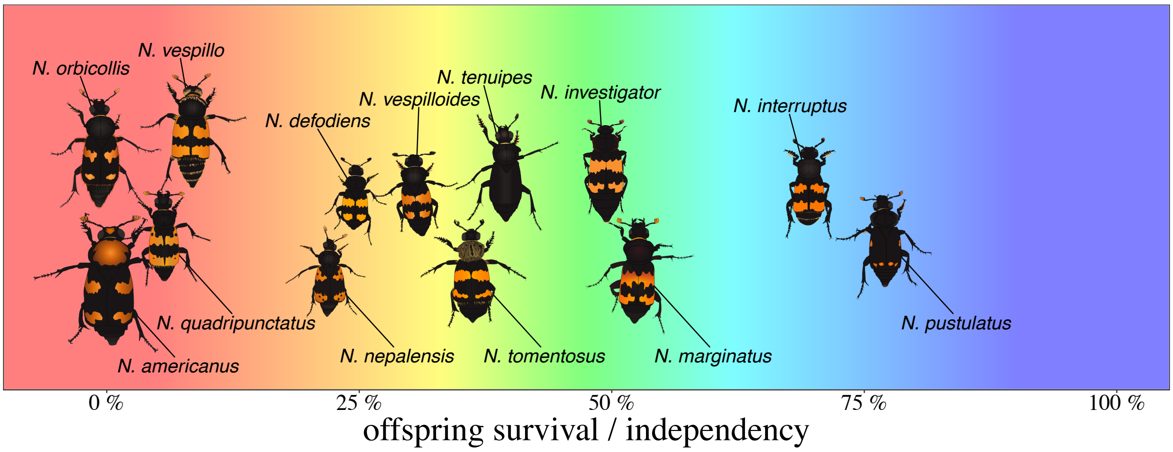


Figure II.
Offspring independency on parental care of different *Nicrophorus* species. Offspring independency was calculated by dividing the surviving larvae of broods without parental care by the initial brood size and subtracting it from 1. Lower offspring survival indicates higher dependency. The pictures of beetles are placed centered on the resulting parameter independency. The beetle graphics are comparable in size to each other (Capodeanu-Nägler, Prang, Steiger et *al. in preparation*).

Our own investigations of interspecific larval traits (**Publication 2: Offspring dependence on parental care and the role of parental transfer of oral fluids in burying beetles**) revealed metabolic differences in burying beetle larvae and subtle evidence for intraspecific variation in larval self-feeding abilities. At the same time, a study by Benowitz *et al.* (2018) highlighted morphological variation in larval mandibles. Their results suggest a possible correlation between larval mandibular serration and larval independence; more specifically, they found that independent species have serration on the inner edge of the mandibles of first instar larvae, whereas dependent species have smooth mandibles with serration developing only in later instars, but their measure of independence is based on personal observations of different researchers (see Jarrett *et al.* 2017). Furthermore, our own comparative study (**Publication 1: Parental feeding and defence of young facilitate faster offspring growth**) found no evidence for a correlation between dependence on parental care and adult body size in burying beetles. Consequently, the results of the study of

Benowitz *et al.* (2018) might need to be re-evaluated. The morphological differences in mandibular serration could also be due to differences in environmental factors, such as soil temperature or humidity, as suggested by Pukowski (1934b).

Regardless of the serration of the larval mandibles, we found sufficient evidence for the importance of oral secretion in explaining larval dependence on parental care in my second study (**Publication 2: Offspring dependence on parental care and the role of parental transfer of oral fluids in burying beetles**). When we provided larvae with a liquidized paste of baby mice, the highly dependent *N. orbicollis* larvae did not survive, but when we added oral fluids of parental *N. orbicollis*, we could significantly increase offspring survival, but not enough to ensure survival to the same level as with full parental care. I therefore decided to focus on parental oral secretions, which are used by parental beetles during post-hatching care for both carcass preservation and direct food provisioning for their offspring. Post-hatching care has been shown to be crucial for ensuring offspring survival in burying beetles (Eggert, Reinking & Müller 1998; Capodeanu-Nägler *et al.* 2016). This post-hatching care encompasses various aspects, and oral secretions contribute to both direct parental care through food provisioning and indirect parental care via carcass modification. Therefore, the benefits of oral secretions cannot be easily assigned to specific parental care behaviors

In species where parental care is multifaceted, disentangling specific parental behaviors and their influence on offspring often becomes a challenge. This is especially true in species where parents use food to build the nest. This complexity is not limited to burying beetles but extends to other species such as the dung beetle *Onticellus cinctus* and the foliage spider *Chiracanthium japonicum*. In *Onticellus cinctus,* the mother provides indirect care by digging a nest and constructing a ball out of dung, the so called 'brood ball' for each egg. Without the food resource itself or the maternal maintenance of the brood ball's integrity, these larvae risk an increased risk of mortality (Halffter & Matthews 1966; Klemperer 1983). Similarly, in the foliage spider *Chiracanthium japonicum*, the mother provides indirect care by building a nest for the eggs and provides direct care in the form of matriphagy (self-sacrificial food provisioning; Toyama 1999). Removal of the mother led to a decrease in hatchling emergence rates because hatchlings are not provided with food and thus leave the nest at an earlier instar. Removing the nest led to a further decline in egg survival (Toyama 1999). Increasing the duration of staying in the nest and providing sufficient food resources for offspring, would likely reduce offspring mortality in such species. This can be supported by observations that enhancements in life-history traits like egg size and incubation time have been shown to decrease offspring mortality, as they allow parents to reduce the proportion of time that offspring spend in the relatively 'high-risk' juvenile stage (Shine 1989; Klug, Alonzo & Bonsall 2012).

In our second study (**Publication 2: Offspring dependence on parental care and the role of parental transfer of oral fluids in burying beetles**), I found that in the dependent burying beetle *N. orbicollis*, only 3 h of post-hatching care was sufficient to significantly increase larval survival. When this period was extended to 12 h of post-hatching care, larval performance was comparable to that of larvae that received the full amount of care. Note that *N. orbicollis* parents typically care for their offspring until the larvae disperse at about 120 h after hatching. Thus, only a short period of parental care appears to be critical for larval survival. However, it seems unlikely that such a short period of care can provide enough nutrients for survival; rather, we suggest that the oral secretions that parents transfer to their offspring may contain enzymes or growth hormones, similar to what is observed in ants (LeBoeuf *et al.* 2016; LeBoeuf 2017).

In burying beetles, studies have shown that parental secretions contain beneficial microbes. These microbes are inoculated into the feeding cavity by the parents (Hall *et al.* 2011; Shukla, Plata*, et al.* 2018; Shukla, Vogel*, et al.* 2018) and play a role in facilitating larval self-feeding (Trumbo 1992; Eggert, Reinking & Müller 1998; Capodeanu-Nägler *et al.* 2016; Shukla, Plata*, et al.* 2018). In the case of the burying beetle species *N. orbicollis*, these essential microbes might be transferred via oral and anal secretions within the first 3 hours of parental care. A parallel can be drawn with the wood-feeding cockroach *Cryptocercus punctulatus*. Here, parental care involves the provision of hindgut fluids containing nutrients and cellulose-digesting symbionts to the offspring (Cleveland 1934; Nalepa 1990). These symbionts are required for the digestion of wood, which is the primary food source for the cockroach. Offspring that do not receive parental provision before reaching the third instar face an increased mortality (Kitade 1997; Inoue *et al.* 2000; Nalepa 2015).

Similarly, in the burying beetle *N. vespilloides*, larvae that develop in nests that have not been modified by their parents, miss out on the vertically transferred parental microbes, especially the beneficial yeast *Yarrowia* (see e.g., Kaltenpoth & Steiger 2014; Vogel *et al.* 2017; Shukla, Plata*, et al.* 2018; Shukla, Vogel*, et al.* 2018; Heise *et al.* 2019; Brinkrolf *et al.* 2021), inheriting instead less advantageous carcass-borne microbes (Wang & Rozen 2018; Miller *et al.* 2019). Although the beneficial transmission of the microbes has been thus far confirmed exclusively through anal secretions in *N. vespilloides* (Kaltenpoth & Steiger 2014; Vogel *et al.* 2017; Shukla, Plata*, et al.* 2018), it might be possible that parental oral secretions benefit larvae not only by the provision of pre-digested food, but also by the beneficial microbes and the previously suggested enzymes or growth hormones. Especially the results of my second study (**Publication 2: Offspring dependence**

on parental care and the role of parental transfer of oral fluids in burying beetles) provided strong evidence of the importance of oral secretions for the survival of burying beetle larvae.

Those oral secretions are however, used for carcass modification and food provisioning, and thus may comprise distinct components tailored for each of those purposes. This intriguing parental oral secretions, divided into the two main behaviors of parental care - food provisioning and carcass modification - became the focus of my third study (**Publication 3: From nutritious nests to parental provisioning: Unveiling the intricate balance of direct and indirect parental care in a highly dependent system**). I addressed this question by manipulating the duration of these two distinct types of care. Our goal was to determine whether the shorter duration of required care in the previous study could be attributed to either indirect or direct care. I found that larval survival was highest when larvae were exposed to at least 12 hours of direct care or a combination of direct and indirect care, while shorter durations of care or only indirect care, i.e., parental modification of the nest, resulted in high larval mortality. Direct care has a greater benefit to larval growth and survival compared to indirect care. Thus, parental regurgitation is critical for offspring survival, but post-hatching manipulation of the food resource is also beneficial to larval performance.

In natural broods, parents are not the only ones to modify the carcass and especially the feeding cavity. In my second study (**Publication 2: Offspring dependence on parental care and the role of parental transfer of oral fluids in burying beetles**), I was able to show that burying beetle larvae can, at least to some extent, feed themselves from the carcass regardless of their dependence on parental care (see Capodeanu-Nägler *et al.* 2018). Therefore, it is reasonable to assume that larvae might also be capable to manipulate the feeding cavity to facilitate food uptake. Therefore, I decided to investigate the presence and potential benefits of sibling interactions in burying beetles in my fourth study. In this study (**Publication 4: Differences in sibling cooperation in presence and absence of parental care in a genus with interspecific variation in offspring dependence**), I was able to show that sibling cooperation occurs in burying beetles. This sibling cooperation is not only influenced by environmental conditions but also by the degree of offspring dependence on parental care. We suggest that the benefits larvae derive from developing with conspecific larvae may be mediated by shared costs of digestive, social immunity-related, and/or thermal effects. Although all species benefited from developing with conspecific larvae under harsh environmental conditions (in the absence of prehatch care), only the most independent species benefited from developing with conspecific larvae under more benign environmental conditions, i.e., in the presence of prehatch care. This is consistent with previous research on *N. vespilloides*, which has shown that harsh environmental conditions tend to promote mutually beneficial interactions between siblings (Schrader, Jarrett & Kilner 2015a). In contrast, more benign conditions, where parental care is present prior to hatching, appear to promote competition rather than cooperation among larvae (Smiseth, Lennox & Moore 2007). This observation is not unique to burying beetles; it has also been observed in other subsocial insects. For example, studies of the European earwig (*Forficula auricularia*) have shown that sibling cooperation is enhanced in scenarios where parental care is either poor or entirely absent (Falk *et al.* 2014). Thus, our results further enhance our understanding of the role of social processes as drivers in the evolution of family life.

In summary, my studies contribute to the knowledge about the complex and dynamic nature of parental care evolution in burying beetles. While my first study (**Publication 1: Parental feeding and defence of young facilitate faster offspring growth**) found no significant relationship between offspring dependency and life-history traits, such as adult body size and egg size, I want to emphasize that ongoing research (Capodeanu-Nägler, Prang, and Steiger *et al.*, in preparation) may reveal differences in parent-offspring interactions. A previous study by Capodeanu-Nägler *et al.* (2017) demonstrated that dependent offspring exhibit increased begging behavior toward their parents compared to independent offspring, and subsequently parents of dependent offspring respond to begging by providing food more often than parents of independent offspring. I could also show, that in burying beetles, parental oral secretions play an important role in explaining offspring dependence on parental care, as shown in my second (**Publication 2: Offspring dependence on parental care and the role of parental transfer of oral fluids in burying beetles**) and third (**Publication 3: From nutritious nests to parental provisioning: Unveiling the intricate balance of direct and indirect parental care in a highly dependent system**) study. However, the exact components of parental oral secretions in burying beetles that cause intraspecific differences in offspring dependence remain unclear. I therefore encourage further comparative studies to analyze the components of parental oral fluids in dependent and independent species. I also encourage comparative studies that examine the influence of further social processes, specifically, sibling interactions seem to play a role in the evolution of family life in burying beetles, as shown in my fourth study (**Publication 4: Differences in sibling cooperation in presence and absence of parental care in a genus with interspecific variation in offspring dependence**). Further family interactions between offspring and parents, and between parents themselves, may also play a role in the emergence and maintenance of family life in burying beetles.

References

Alonso-Alvarez, C. & Velando, A. (2012) Benefits and costs of parental care. *The evolution of parental care* (eds N.J. Royle, P.T. Smiseth & M. Kölliker), pp. 40-61. Oxford University Press, Oxford, U.K., https://doi.org/10.1093/acprof:oso/9780199692576.003.0003

Anderson, R.S. (1982) Burying beetle larvae: Nearctic *Nicrophorus* and Oriental *Ptomascopus morio* (Silphidae). *Systematic Entomology,* 7, 249-264. https://doi.org/10.1111/j.1365-3113.1982.tb00443.x

Anduaga, S. (2009) Reproductive biology of *Nicrophorus mexicanus* Matthews (Coleoptera: Silphidae). *The Coleopterists Bulletin,* 63, 173-178. https://doi.org/10.1649/1034.1

Arce, A.N., Johnston, P.R., Smiseth, P.T. & Rozen, D.E. (2012) Mechanisms and fitness effects of antibacterial defences in a carrion beetle. *Journal of Evolutionary Biology,* 25, 930-937. https://doi.org/10.1111/j.1420-9101.2012.02486.x

Ballard, O. & Morrow, A.L. (2013) Human milk composition: Nutrients and bioactive factors. *Pediatric clinics of North America,* 60, 49-74. https://doi.org/10.1016/j.pcl.2012.10.002

Balshine, S. (2012) Patterns of parental care in vertebrates. *The evolution of parental care* (eds N.J. Royle, P.T. Smiseth & M. Kölliker), pp. 62-80. Oxford Universtiy Press, Oxford, U.K., https://doi.org/10.1093/acprof:oso/9780199692576.003.0004

Bartlett, J. (1987a) Evidence for sex attractant in burying beetles. *Ecological Entomology,* 12, 471-472.

Bartlett, J. (1987b) Filial cannibalism in burying beetles. *Behavioral Ecology and Sociobiology,* 21, 179-183.

Baskett, J.N. (1897) *The story of the birds*. D. Appleton and Company, New York, USA,

Baur, B. (1994) Parental care in terrestrial gastropods. *Experientia,* 50, 5-14. https://doi.org/10.1007/BF01992042

Benowitz, K.M., Sparks, M.E., McKinney, E.C., Moore, P.J. & Moore, A.J. (2018) Variation in mandible development and its relationship to dependence on parents across burying beetles. *Ecology and Evolution,* 8, 12832-12840. https://doi.org/10.1002/ece3.4713

Boggs, C.L. (2009) Understanding insect life histories and senescence through a resource allocation lens. *Functional Ecology,* 23, 27-37. https://doi.org/10.1111/j.1365-2435.2009.01527.x

Bonsall, M. & Klug, H. (2011) The evolution of parental care in stochastic environments. *Journal of Evolutionary Biology,* 24, 645-655. https://doi.org/10.1111/j.1420-9101.2010.02203.x

Botelho, J.F. & Faunes, M. (2015) The evolution of developmental modes in the new avian phylogenetic tree. *Evolution & Development,* 17, 221-223. https://doi.org/10.1111/ede.12126

Bourke, A.F.G. (2011) *Principles of social evolution*. Oxford University Press, https://doi.org/10.1093/acprof:oso/9780199231157.001.0001

Brinkrolf, K., Shukla, S.P., Griep, S., Rupp, O., Heise, P., Goesmann, A., Heckel, D.G., Vogel, H. & Vilcinskas, A. (2021) Genomic analysis of novel *Yarrowia*-like yeast symbionts associated with the carrion-feeding burying beetle *Nicrophorus vespilloides*. *BMC Genomics,* 22, 323. https://doi.org/10.1186/s12864-021-07597-z

Cai, C., Tihelka, E., Giacomelli, M., Lawrence, J.F., Ślipiński, A., Kundrata, R., Yamamoto, S., Thayer, M.K., Newton, A.F. & Leschen, R.A. (2022) Integrated phylogenomics and fossil data illuminate the evolution of beetles. *Royal Society Open Science,* 9, 211771. https://doi.org/10.1098/rsos.211771

Campos-Cerda, F. & Bohannan, B.J.M. (2020) The nidobiome: A framework for understanding microbiome assembly in neonates. *Trends in Ecology & Evolution,* 35, 573-582. https://doi.org/10.1016/j.tree.2020.03.007

Capodeanu-Nägler, A., Eggert, A.-K., Vogel, H., Sakaluk, S.K. & Steiger, S. (2017) Species divergence in offspring begging and parental provisioning is linked to nutritional dependency. *Behavioral Ecology,* 29, 42-50. https://doi.org/10.1093/beheco/arx117

Capodeanu-Nägler, A., Keppner, E.M., Vogel, H., Ayasse, M., Eggert, A.-K., Sakaluk, S.K. & Steiger, S. (2016) From facultative to obligatory parental care: Interspecific variation in offspring dependency on post-hatching care in burying beetles. *Scientific Reports,* 6, 29323. https://doi.org/10.1038/srep29323

Capodeanu-Nägler, A., Prang, M.A., Trumbo, S.T., Vogel, H., Eggert, A.-K., Sakaluk, S.K. & Steiger, S. (2018) Offspring dependence on parental care and the role of parental transfer of oral fluids in burying beetles. *Frontiers in Zoology,* 15, 1-12. https://doi.org/10.1186/s12983-018-0278-5

Castro, G., Stoyan, N. & Myers, J.P. (1989) Assimilation efficiency in birds: A function of taxon or food type? *Comparative Biochemistry and Physiology Part A: Physiology,* 92A, 271-278. https://doi.org/10.1016/0300-9629(89)90563-X

Cleveland, L.R. (1934) The wood feeding roach *Cryptocercus,* its protozoa, and the symbiosis between protozoa and roach. *Memoirs of the American academy of arts and sciences,* 17, 185-382.

Clutton-Brock, T.H. (1991) *The evolution of parental care*. Princeton University Press, Princeton, New Jersey, USA, https://doi.org/10.1046/j.1420-9101.1992.5040719.x

Costa, J.T. (2006) *The other insect societies*. Harvard University Press, Cambridge, Massachusetts, USA,

Cotter, S.C. & Kilner, R.M. (2010a) Personal immunity versus social immunity. *Behavioral Ecology,* 21, 663-668. https://doi.org/10.1093/beheco/arq070

Cotter, S.C. & Kilner, R.M. (2010b) Sexual division of antibacterial resource defence in breeding burying beetles, *Nicrophorus vespilloides*. *Journal of Animal Ecology,* 79, 35-43. https://doi.org/10.1111/j.1365-2656.2009.01593.x

Creighton, J.C., Smith, A.N., Komendat, A. & Belk, M.C. (2014) Dynamics of biparental care in a burying beetle: Experimental handicapping results in partner compensation. *Behavioral Ecology and Sociobiology,* 69, 265-271. https://doi.org/10.1007/s00265-014-1839-z

Darwin, C. (1871) Chapter III: Comparison of the mental powers of man and the lower animals. *Descent of man, and selection in its relation to sex*. John Murray, London,

Degenkolb, T., Düring, R.-A. & 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. https://doi.org/10.1007/s10886-011-9978-4

Dorsey, C.K. (1940) A comparative study of the larvae of six species of *Silpha*. *Annals of the Entomological Society of America,* 33, 120-139. https://doi.org/10.1093/aesa/33.1.120

Eberhard, W.G. (1975) *The ecology and behavior of a subsocial pentatomid bug and two scelionid wasps: Strategy and counterstrategy in a host and its parasites*. Smithsonian Institution Press, City of Washington, USA,

Eggert, A.-K. (1992) Alternative male mate-finding tactics in burying beetles. *Behavioral Ecology,* 3, 243-254.

Eggert, A.-K. & Müller, J.K. (1992) Joint breeding in female burying beetles. *Behavioral Ecology and Sociobiology,* 31, 237-242.

Eggert, A.-K. & Müller, J.K. (2000) Timing of oviposition and reproductive skew in cobreeding female burying beetles (*Nicrophorus vespilloides*). *Behavioral Ecology,* 11, 357-366. https://doi.org/10.1093/beheco/11.4.357

Eggert, A.-K. & Müller, J.K. (2011) Timing of oviposition enables dominant female burying beetles to destroy brood-parasitic young. *Animal Behaviour,* 82, 1227-1233. https://doi.org/10.1016/j.anbehav.2011.09.001

Eggert, A.-K., Reinking, M. & Müller, J.K. (1998) Parental care improves offspring survival and growth in burying beetles. *Animal Behaviour,* 55, 97-107. https://doi.org/10.1006/anbe.1997.0588

Eggert, A.-K. & Sakaluk, S.K. (2000) Benefits of communal breeding in burying beetles: A field experiment. *Ecological Entomology,* 25, 262-266. https://doi.org/10.1046/j.1365-2311.2000.00262.x

Engberg, R.M., Kaspers, B., Schranner, I., Kösters, J. & Lösch, U. (1992) Quantification of the immunoglobulin classes IgG and IgA in the young and adult pigeon (*Columba livia*). *Avian Pathology,* 21, 409-420. https://doi.org/10.1080/03079459208418859

Engel, K.C., Hwang, W. & Steiger, S. (2018) A pheromone that coordinates parental care is evolutionary conserved among burying beetles (Silphidae: *Nicrophorus*). *Chemoecology*. https://doi.org/10.1007/s00049-018-0271-7

Engel, K.C., Stökl, J., Schweizer, R., Vogel, H., Ayasse, M., Ruther, J. & Steiger, S. (2016) A hormone-related female anti-aphrodisiac signals temporary infertility and causes sexual abstinence to synchronize parental care. *Nature Communications,* 7, 10. https://doi.org/10.1038/ncomms11035

Engel, K.C., von Hoermann, C., Eggert, A.-K., Müller, J.K. & Steiger, S. (2014) When males stop having sex: Adaptive insect mating tactics during parental care. *Animal Behaviour,* 90, 245-253. https://doi.org/10.1016/j.anbehav.2014.02.006

Eraud, C., Dorie, A., Jacquet, A. & Faivre, B. (2008) The crop milk: A potential new route for carotenoid-mediated parental effects. *J Avian Biol,* 39, 247-251. https://doi.org/10.1111/j.0908-8857.2008.04053.x

Falk, J., Wong, J.W.Y., Kölliker, M. & Meunier, J. (2014) Sibling cooperation in earwig families provides insights into the early evolution of social life. *The American Naturalist,* 183, 547-557. https://doi.org/10.1086/675364

Fetherston, I.A., Scott, M.P. & Traniello, J.F.A. (1994) Behavioural compensation for mate loss in the burying beetle *Nicrophorus orbicollis*. *Animal Behaviour,* 47, 777-785.

Freckleton, R.P. & Harvey, P.H. (2006) Detecting non-Brownian trait evolution in adaptive radiations. *PLOS Biology,* 4, e373. https://doi.org/10.1371/journal.pbio.0040373

Gardner, A. & Smiseth, P.T. (2011) Evolution of parental care driven by mutual reinforcement of parental food provisioning and sibling competition. *Proceedings of the Royal Society B: Biological Sciences,* 278, 196-203. https://doi.org/10.1098/rspb.2010.1171

Goldman, A. & Chheda, S. (2021) The immune system in human milk: A historic perspective. *Annals of Nutrition and Metabolism,* 77, 1-8. https://doi.org/10.1159/000516995

Goldman, A.S. (1993) The immune system of human milk: Antimicrobial, antiinflammatory and immunomodulating properties. *The Pediatric Infectious Disease Journal,* 12, 664-672.

Grubbauer, P. & Hoi, H. (1996) Female penduline tits (*Remiz pendulinus*) choosing high quality nests benefit by decreased incubation effort and increased hatching success. *Écoscience,* 3, 274-279. https://doi.org/10.1080/11956860.1996.11682342

Gutiérrez, R., Córdova-Lepe, F., Moreno-Gómez, F.N. & Velásquez, N.A. (2020) Persistence and size of seasonal populations on a consumer–resource relationship depends on the allocation strategy toward life-history functions. *Scientific Reports,* 10, 21401. https://doi.org/10.1038/s41598-020-77326-1

Halffter, G. & Matthews, E.G. (1966) *The natural history of dung beetles of the subfamily Scarabaeinae (Coleoptera, Scarabaeidae)*. Sociedad Mexicana de Entomología, Mexico City, Mexico,

Hall, C.L., Wadsworth, N.K., Howard, D.R., Jennings, E.M., Farrell, L.D., Magnuson, T.S. & Smith, R.J. (2011) Inhibition of microorganisms on a carrion breeding resource: The antimicrobial peptide activity of burying beetle (Coleoptera: Silphidae) oral and anal secretions. *Environmental Entomology,* 40, 669-678. https://doi.org/10.1603/EN10137

Heise, P., Liu, Y., Degenkolb, T., Vogel, H., Schäberle, T.F. & Vilcinskas, A. (2019) Antibiotic-producing beneficial bacteria in the gut of the burying beetle *Nicrophorus vespilloides*. *Frontiers in Microbiology,* 10. https://doi.org/10.3389/fmicb.2019.01178

Hoback, W.W., Bishop, A.A., Kroemer, J., Scalzitti, J. & Shaffer, J.J. (2004) Differences among antimicrobial properties of carrion beetle secretions reflect phylogeny and ecology. *Journal of Chemical Ecology,* 30, 719-729. https://doi.org/10.1023/b:joec.0000028427.53141.41

Hocking, M.D., Darimont, C.T., Christie, K.S. & Reimchen, T.E. (2007) Niche variation in burying beetles (*Nicrophorus* spp.) associated with marine and terrestrial carrion. *Canadian Journal of Zoology,* 85, 437-442. https://doi.org/10.1139/z07-016

Hocking, M.D., Ring, R.A. & Reimchen, T.E. (2006) Burying beelte *Nicrophorus investigator* reproduction on Pacific salmon carcasses. *Ecological Entomology,* 31, 5-12. https://doi.org/10.1111/j.0307-6946.2006.00747.x

Inoue, T., Kitade, O., Yoshimura, T. & Yamaoka, I. (2000) Symbiotic associations with protists. *Termites: Evolution, sociality, symbioses, ecology* (eds T. Abe, D.E. Bignell & M. Higashi), pp. 275-288. Springer Dordrecht, https://doi.org/10.1007/978-94-017-3223-9_13

Jacobs, C.G.C., Steiger, S., Heckel, D.G., Wielsch, N., Vilcinskas, A. & Vogel, H. (2016) Sex, offspring and carcass determine antimicrobial peptide expression in the burying beetle. *Scientific Reports,* 6, 25409. https://doi.org/10.1038/srep25409

Jarrett, B.J., Schrader, M., Rebar, D., Houslay, T.M. & Kilner, R.M. (2017) Cooperative interactions within the family enhance the capacity for evolutionary change in body size. *Nature Ecology & Evolution,* 1, 0178. https://doi.org/10.1038/s41559-017-0178

Jetz, W., Sekercioglu, C.H. & Böhning-Gaese, K. (2008) The worldwide variation in avian clutch size across species and space. *PLOS Biology,* 6, e303. https://doi.org/10.1371/journal.pbio.0060303

Kaltenpoth, M., Göttler, W., Herzner, G. & Strohm, E. (2005) Symbiotic bacteria protect wasp larvae from fungal infestation. *Current Biology,* 15, 475-479. https://doi.org/10.1016/j.cub.2004.12.084

Kaltenpoth, M. & Steiger, S. (2014) Unearthing carrion beetles' microbiome: Characterization of bacterial and fungal hindgut communities across the Silphidae. *Molecular Ecology,* 23, 1251-1267. https://doi.org/10.1111/mec.12469

Karasov, W.H. (1990) Digestion in birds: Chemical and physiological determinants and ecological implications. *Studies in avian biology,* 13, 391-415.

Kim, K.W., Krafft, B. & Choe, J.C. (2005) Cooperative prey capture by young subsocial spiders. *Behavioral Ecology and Sociobiology,* 59, 92-100. https://doi.org/10.1007/s00265-005-0013-z

Kitade, O. (1997) Establishment of symbiotic flagellate fauna of *Hodotermopsis japonica* (Isoptera: Termopsidae). *Sociobiology,* 30, 161.

Kleiman, D.G. & Malcolm, J.R. (1981) The evolution of male parental investment in mammals. *Parental care in mammals*, pp. 347-387. Springer,

Klemperer, H.G. (1983) Subsocial behaviour in *Oniticellus cinctus* (Coleoptera, Scarabaeidae): Effect of the brood on parental care and oviposition. *Physiological Entomology,* 8, 393-402.

Klug, H., Alonzo, S.H. & Bonsall, M.B. (2012) Theoretical foundations of parental care. *The evolution of parental care* (eds N.J. Royle, P.T. Smiseth & M. Kölliker), pp. 21-36. Oxford University Press, Oxford, U.K., https://doi.org/10.1093/acprof:oso/9780199692576.003.0002

Klug, H. & Bonsall, M.B. (2009) Life history and the evolution of parental care. *Evolution,* 64, 823-835. https://doi.org/10.1111/j.1558-5646.2009.00854.x

Klug, H. & Bonsall, M.B. (2014) What are the benefits of parental care? The importance of parental effects on developmental rate. *Ecology and Evolution,* 4, 2330-2351. https://doi.org/10.1002/ece3.1083

Kölliker, M. (2007) Benefits and costs of earwig (*Forficula auricularia*) family life. *Behavioral Ecology and Sociobiology* 61, 1489-1497. https://doi.org/10.1007/s00265-007-0381-7

Kramer, J. & Meunier, J. (2019) The other facets of family life and their role in the evolution of animal sociality. *Biological Reviews,* 94, 199-215. https://doi.org/10.1111/brv.12443

Kramer, J., Thesing, J. & Meunier, J. (2015) Negative association between parental care and sibling cooperation in earwigs: A new perspective on the early evolution of family life? *Journal of Evolutionary Biology,* 28, 1299-1308. https://doi.org/10.1111/jeb.12655

Krause, J. & Ruxton, G.D. (2002) *Living in groups*. Oxford University Press, ISBN: 9780198508182

Lahti, D.C., Johnson, N.A., Ajie, B.C., Otto, S.P., Hendry, A.P., Blumstein, D.T., Coss, R.G., Donohue, K. & Foster, S.A. (2009) Relaxed selection in the wild. *Trends in Ecology & Evolution,* 24, 487-496. https://doi.org/10.1016/j.tree.2009.03.010

LeBoeuf, A.C. (2017) Trophallaxis. *Current Biology Magazine,* 27, R1299-R1300. https://doi.org/10.1016/j.cub.2017.10.047

LeBoeuf, A.C., Waridel, P., Brent, C.S., Gonçalves, A.N., Menin, L., Ortiz, D., Riba-Grognuz, O., Koto, A., Soares, Z.G., Privman, E., Miska, E.A., Benton, R. & Keller, L. (2016) Oral transfer of chemical cues, growth proteins and hormones in social insects. *Elife,* 5. https://doi.org/10.7554/eLife.20375

Maekawa, K., Matsumoto, T. & Nalepa, C.A. (2008) Social biology of the wood-feeding cockroach genus *Salganea* (Dictyoptera, Blaberidae, Panesthiinae): Did ovoviviparity prevent the evolution of eusociality in the lineage? *Insectes Sociaux,* 55, 107-114. https://doi.org/10.1007/s00040-008-0997-2

Magneville, C., Ratz, T., Richardson, J. & Smiseth, P.T. (2018) No evidence of sibling cooperation in the absence of parental care in *Nicrophorus vespilloides*. *Evolution,* 72, 2803-2809. https://doi.org/10.1111/evo.13622

Martin, R.D. & MacLarnon, A.M. (1985) Gestation period, neonatal size and maternal investment in placental mammals. *Nature,* 313, 220-223. https://doi.org/10.1038/313220a0

Mennerat, A., Mirleau, P., Blondel, J., Perret, P., Lambrechts, M.M. & Heeb, P. (2009) Aromatic plants in nests of the blue tit *Cyanistes caeruleus* protect chicks from bacteria. *Oecologia,* 161, 849-855. https://doi.org/10.1007/s00442-009-1418-6

Meunier, J., Körner, M. & Kramer, J. (2022) Parental care. *Reproductive Strategies in Insects*, pp. 337-357. https://doi.org/10.1201/9781003043195-17

Miller, C.J., Bates, S.T., Gielda, L.M. & Creighton, J.C. (2019) Examining transmission of gut bacteria to preserved carcass via anal secretions in *Nicrophorus defodiens*. *PLoS One,* 14, e0225711. https://doi.org/10.1371/journal.pone.0225711

Müller, J.K. (1987) Replacement of a lost clutch: A strategy for optimal resource utilization in *Necrophorus vespilloides (*Coleoptera: Silphidae). *Ethology,* 76, 74-80.

Müller, J.K., Braunisch, V., Hwang, W. & Eggert, A.-K. (2007) Alternative tactics and individual reproductive success in natural associations of the burying beetle, *Nicrophorus vespilloides*. *Behavioral Ecology,* 18, 196-203. https://doi.org/10.1093/beheco/arl073

Müller, J.K. & Eggert, A.-K. (1990) Time-dependent shifts between infanticidal and parental behavior in female burying beetles: A mechanism of indirect mother-offspring recognition. *Behavioral Ecology and Sociobiology,* 27, 11-16. https://doi.org/10.1007/BF00183307

Müller, J.K., Eggert, A.-K. & Furlkröger, E. (1990) Clutch size regulation in the burying beetle *Necrophorus vespilloides* Herbst (Coleoptera: Silphidae). *Journal of Insect Behavior,* 3, 265-270. https://doi.org/10.1007/BF01417917

Nalepa, C., Bignell, D. & Bandi, C. (2001) Detritivory, coprophagy, and the evolution of digestive mutualisms in Dictyoptera. *Insectes Sociaux,* 48, 194-201. https://doi.org/10.1007/PL00001767

Nalepa, C.A. (1990) Early development of nymphs and establishment of hindgut symbiosis in *Cryptocercus punctulatus* (Dictyoptera: Cryptocercidae). *Annals of the Entomological Society of America,* 83, 786-789. https://doi.org/10.1093/aesa/83.4.786

Nalepa, C.A. (2015) Origin of termite eusociality: Trophallaxis integrates the social, nutritional, and microbial environments. *Ecological Entomology,* 40, 323-335. https://doi.org/10.1111/een.12197

Nalepa, C.A., Maekawa, K., Shimada, K., Saito, Y., Arellano, C. & Matsumoto, T. (2008) Altricial development in subsocial wood-feeding cockroaches. *Zoological Science,* 25, 1190-1198. https://doi.org/10.2108/zsj.25.1190

Otronen, M. (1988) The effect of body size on the outcome of fights in burying beetles. *Annales Zoologici Fennici,* 25, 191-201.

Prang, M.A., Zywucki, L., Körner, M. & Steiger, S. (2022) Differences in sibling cooperation in presence and absence of parental care in a genus with interspecific variation in offspring dependence. *Evolution,* 76, 320-331. https://doi.org/10.1111/evo.14414

Pukowski, E. (1933) Ökologische Untersuchungen an *Necrophorus* F. *Zeitschrift für Morphologie und Ökologie der Tiere,* 27, 518-586. https://doi.org/10.1007/BF00403155

Pukowski, E. (1934a) Die Brutpflege des Totengräbers. *Entomologische Blätter,* 30, 109-113.

Pukowski, E. (1934b) Zur Systematik der *Necrophorus*-Larven (Col.). *Stett Ent Ztg,* 95, 53-.

Rahn, H., Paganelli, C. & Ar, A. (1975) Relation of avian egg weight to body weight. *The Auk,* 92, 750-765.

Ratcliffe, B.C. (1996) The carrion beetles (Coleoptera: Silphidae) of Nebraska. *Bulletin of the University of Nebraska State Museum,* 13, 1-107. https://doi.org/10.1093/aesa/90.3.399

Rauter, C.M. & Moore, A.J. (1999) Do honest signalling models of offspring solicitation apply to insects? *Proceedings of the Royal Society B: Biological Sciences,* 266, 1691. https://doi.org/10.1098/rspb.1999.0833

Rebar, D., Bailey, N.W., Jarrett, B.J.M. & Kilner, R.M. (2020) An evolutionary switch from sibling rivalry to sibling cooperation, caused by a sustained loss of parental care. *Proceedings of the National Academy of Sciences,* 117, 2544-2550. https://doi.org/10.1073/pnas.1911677117

Robertson, I.C. (1992) Relative abundance of *Nicrophorus pustulatus* (Coleoptera: Silphidae) in a burying beetle community, with notes on its reproductive behavior. *Psyche: A Journal of Entomology,* 99, 189-198.

Roff, D.A. (1993) *Evolution of life histories: Theory and analysis*. Springer Science & Business Media, 1993, New York, U.S.A., ISBN: 9780412023910

Royle, N.J., Smiseth, P.T. & Kölliker, M. (2012) *The evolution of parental care*. Oxford University Press, Oxford, U.K., https://doi.org/10.1093/acprof:oso/9780199692576.001.0001

Rozen, D.E., Engelmoer, D.J.P. & Smiseth, P.T. (2008) Antimicrobial strategies in burying beetles breeding on carrion. *Proceedings of the National Academy of Sciences of the United States of America,* 105, 17890-17895. https://doi.org/10.1073/pnas.0805403105

Ruiz-Castellano, C., Tomás, G., Ruiz-Rodríguez, M., Martín-Gálvez, D. & Soler, J.J. (2016) Nest material shapes eggs bacterial environment. *PLoS One,* 11, e0148894. https://doi.org/10.1371/journal.pone.0148894

Růžička, J., Háva, J.i. & Haberer, W. (2000) Toxonomical and distributional notes on Oriental Silphidae, with description of *Nicrophorus sausai* sp.n. (Insecta: Coleoptera). *Reichenbachia,* 33, 377-384.

Růžička, J., Jakubec, P., Mahlerová, K., Šípková, H. & Nishikawa, M. (2023) Integrative taxonomy and species distribution models of the genus *Diamesus* Hope, 1840 (Coleoptera: Staphylinidae: Silphinae). *Scientific Reports,* 13, 3192. https://doi.org/10.1038/s41598-023-30019-x

Sahm, J., Conrad, T., Scheu, L. & Steiger, S. (2023) Brood size, food availability, and body size affects male care decisions and offspring performance. *Ecology and Evolution,* 13, e10183. https://doi.org/10.1002/ece3.10183

Sahm, J., Prang, M.A. & Steiger, S. (2022) Parent–offspring conflict and its outcome under uni-and biparental care. *Scientific Reports,* 12, 1999. https://doi.org/10.1038/s41598-022-05877-6

Schrader, M., Jarrett, B.J. & Kilner, R.M. (2015a) Parental care masks a density-dependent shift from cooperation to competition among burying beetle larvae. *Evolution,* 69, 1077-1084. https://doi.org/10.1111/evo.12615

Scott, M.P. (1997) Reproductive dominance and differential ovicide in the communally breeding burying beetle *Nicrophorus tomentosus*. *Behavioral Ecology and Sociobiology,* 40, 313-320. https://doi.org/10.1007/s002650050347

Scott, M.P. & Traniello, J.F.A. (1987) Behavioural cues trigger ovarian development in the burying beetle, *Nicrophorus tomentosus*. *Journal of Insect Physiology,* 33, 693-696. https://doi.org/10.1016/0022-1910(87)90053-9

Scott, M.P. & Traniello, J.F.A. (1990) Behavioural and ecological correlates of male and female parental care and reproductive success in burying beetles (*Nicrophorus* spp.). *Animal Behaviour,* 39, 274-283. https://doi.org/10.1016/S0003-3472(05)80871-1

Shetty, S., Bharathi, L., Shenoy, K.B. & Hegde, S.N. (1992) Biochemical properties of pigeon milk and its effect on growth. *J Comp Physiol B,* 162, 632-636. https://doi.org/10.1007/bf00296644

Shimada, K. & Maekawa, K. (2011) Description of the basic features of parent-offspring stomodeal trophallaxis in the subsocial wood-feeding cockroach *Salganea esakii* (Dictyoptera, Blaberidae, Panesthiinae). *Entomological Science,* 14, 9-12. https://doi.org/10.1111/j.1479-8298.2010.00406.x

Shine, R. (1989) Alternative models for the evolution of offspring size. *The American Naturalist,* 134, 311-317. https://doi.org/10.1086/284982

Shukla, S.P., Plata, C., Reichelt, M., Steiger, S., Heckel, D.G., Kaltenpoth, M., Vilcinskas, A. & Vogel, H. (2018) Microbiome-assisted carrion preservation aids larval development in a burying beetle. *Proceedings of the National Academy of Sciences,* 115, 11274-11279. https://doi.org/10.1073/pnas.1812808115

Shukla, S.P., Vogel, H., Heckel, D.G., Vilcinskas, A. & Kaltenpoth, M. (2018) Burying beetles regulate the microbiome of carcasses and use it to transmit a core microbiota to their offspring. *Molecular Ecology,* 27, 1980-1991. https://doi.org/10.1111/mec.14269

Sikes, D.S., Madge, R.B. & Newton, A.F. (2002) A catalog of the Nicrophorinae (Coleoptera: Silphidae) of the world. *Zootaxa,* 65, 1-304. https://doi.org/10.11646/zootaxa.65.1.1

Sikes, D.S., Madge, R.B. & Trumbo, S.T. (2006) Revision of *Nicrophorus* in part, new species and inferred phylogeny of the *nepalensis*-group based on evidence from morphology and mitochondrial DNA. *Invertebrate Systematics,* 20, 305-365. https://doi.org/10.1071/IS05020

Sikes, D.S. & Mousseau, T. (2013) Description of *Nicrophorus efferens*, new species, from Bougainville Island (Coleoptera, Silphidae, Nicrophorinae). *Zookeys*, 83-93. https://doi.org/10.3897/zookeys.311.5141

Sikes, D.S. & Peck, S.B. (2000) Description of *Nicrophorus hispaniola*, new species, from Hispaniola (Coleoptera: Silphidae) and a key to the species of *Nicrophorus* of the New World. *Annals of the Entomological Society of America,* 93, 391-397.

Sikes, D.S., Trumbo, S.T. & Peck, S.B. (2016) Cryptic diversity in the New World burying beetle fauna: *Nicrophorus hebes* Kirby; new status as a resurrected name (Coleoptera: Silphidae: Nicrophorinae). *Arthropod Syst Phylogeny,* 74, 299-309. https://doi.org/10.3897/asp.74.e31872

Sikes, D.S. & Venables, C. (2013) Molecular phylogeny of the burying beetles (Coleoptera: Silphidae: Nicrophorinae). *Molecular Phylogenetics and Evolution,* 69, 552-565. https://doi.org/10.1016/j.ympev.2013.07.022

Smiseth, P.T., Darwell, C.T. & Moore, A.J. (2003) Partial begging: An empirical model for the early evolution of offspring signalling. *Proceedings of the Royal Society B: Biological Sciences,* 270, 1773-1777. https://doi.org/10.1098/rspb.2003.2444

Smiseth, P.T., Kölliker, M. & Royle, N.J. (2012) What is parental care? *The evolution of parental care* (eds N.J. Royle, P.T. Smiseth & M. Kölliker), pp. 01-17. Oxford University Press, Oxford, U.K., https://doi.org/10.1093/acprof:oso/9780199692576.003.0001

Smiseth, P.T., Lennox, L. & Moore, A.J. (2007) Interaction between parental care and sibling competition: Parents enhance offspring growth and exacerbate sibling competition. *Evolution,* 61, 2331-2339. https://doi.org/10.1111/j.1558-5646.2007.00192.x

Smiseth, P.T., Musa, S. & Moore, A.J. (2006) Negotiation between parents: Does the timing of mate loss affect female compensation in *Nicrophorus vespilloides*? *Behaviour,* 143, 293-301. https://doi.org/10.1163/156853906775897888

Stearns, S.C. (1992) *The evolution of life histories*. Oxford University Press, Oxford, U.K., ISBN: 9780198577416

Steiger, S., Gershman, S.N., Pettinger, A.M., Eggert, A.-K. & 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. https://doi.org/10.1111/j.1365-2435.2011.01895.x

Suzuki, S. (1999) Does carrion-burial by *Nicrophorus vespilloides* (Silphidae: Coleoptera) prevent discovery by other burying beetles? *Entomological Science,* 2, 205-208. ISSN: 13438786

Suzuki, S. (2009) Mate choice and copulation frequency in the burying Beetle *Nicrophorus quadripunctatus* (Coleoptera: Silphidae): Effect of male body size and presence of a rival. *Psyche: A Journal of Entomology,* 2009, 1-4. https://doi.org/10.1155/2009/904604

Suzuki, S. (2016) When the male determines his provisioning effort: Does the timing of handicapping affect the negotiation between parents in *Nicrophorus quadripunctatus*? *Behaviour,* 153, 1435-1443. https://doi.org/10.1163/1568539x-00003389

Szathmáry, E. & Smith, J.M. (1995) The major evolutionary transitions. *Nature,* 374, 227-232. https://doi.org/10.1038/374227a0

Tallamy, D.W. & Wood, T.K. (1986) Convergence patterns in subsocial insects. *Annual Review of Entomology,* 31, 369-390.

Tarwater, C.E. & Brawn, J.D. (2010) The post-fledging period in a tropical bird: Patterns of parental care and survival. *J Avian Biol,* 41, 479-487. https://doi.org/10.1111/j.1600-048X.2010.05006.x

Toyama, M. (1999) Adaptive advantages of maternal care and matriphagy in a foliage spider, *Chiracanthium japonicum* (Araneae: Coubionidae). *Journal of Ethology,* 17, 33-39. https://doi.org/10.1007/BF02769295

Trivers, R.L. (1972) Parental investment and sexual selection. *Sexual selection and the descent of man* (ed. B. Campbell), pp. 136-179. Aldine Publishing Company, Chicago, ISBN: 9780202308456

Trumbo, S.T. (1992) Monogamy to communal breeding: Exploitation of a broad resource base by burying beetles (*Nicrophorus*). *Ecological Entomology,* 17, 289-298. https://doi.org/10.1111/j.1365-2311.1992.tb01060.x

Trumbo, S.T. (2006) Infanticide, sexual selection and task specialization in a biparental burying beetle. *Animal Behaviour,* 72, 1159-1167. https://doi.org/10.1016/j.anbehav.2006.05.004

Trumbo, S.T. (2012) Patterns of parental care in invertebrates. *The evolution of parental care* (eds N.J. Royle, P.T. Smiseth & M. Kölliker), pp. 81-100. Oxford University Press, Oxford, U.K., https://doi.org/10.1093/acprof:oso/9780199692576.003.0005

Trumbo, S.T. & Sikes, D.S. (2021) Resource concealment and the evolution of parental care in burying beetles. *Journal of Zoology,* 315, 175-182. https://doi.org/10.1111/jzo.12916

Vogel, H., Shukla, S.P., Engl, T., Weiss, B., Fischer, R., Steiger, S., Heckel, D.G., Kaltenpoth, M. & Vilcinskas, A. (2017) The digestive and defensive basis of carcass utilization by the burying beetle and its microbiota. *Nature Communications,* 8, 1-15. https://doi.org/10.1038/ncomms15186

Wang, Y. & Rozen, D.E. (2018) Gut microbiota in the burying beetle, *Nicrophorus vespilloides*, provide colonization resistance against larval bacterial pathogens. *Ecology and Evolution,* 8, 1646-1654. https://doi.org/10.1002/ece3.3589

Webb, J.N., Székely, T., Houston, A.I. & McNamara, J.M. (2002) A theoretical analysis of the energetic costs and consequences of parental care decisions. *Philosophical Transactions of the Royal Society B: Biological Sciences* 357, 331-340. https://doi.org/10.1098/rstb.2001.0934

Wesołowski, T. (1994) On the origin of parental care and the early evolution of male and female parental roles in birds. *The American Naturalist,* 143, 39-58. https://doi.org/10.1086/285595

West, S.A., Griffin, A.S. & Gardner, A. (2007b) Social semantics: Altruism, cooperation, mutualism, strong reciprocity and group selection. *Journal of Evolutionary Biology,* 20, 415-432. https://doi.org/10.1111/j.1420-9101.2006.01258.x

Williams, T.D. (1994) Intraspecific variation in egg size and egg composition in birds: Effects on offspring fitness. *Biological Reviews of the Cambridge Philosophical Society,* 69, 35-59. https://doi.org/10.1111/j.1469-185x.1994.tb01485.x

Wilson, D.S. (1975) The adequacy of body size as a niche difference. *The American Naturalist,* 109, 769-784. https://doi.org/10.1086/283042

Wilson, D.S. & Fudge, J. (1984) Burying beetles: Intraspecific interactions and reproductive success in the field. *Ecological Entomology,* 9, 195-203. **https://doi.org/**10.1111/j.1365-2311.1984.tb00715.x

Wilson, D.S. & Knollenberg, W.G. (1984) Food discrimination and ovarian development in burying beetles (Coleoptera: Silphidae: *Nicrophorus*). *Annals of the Entomological Society of America,* 77, 165-170. https://doi.org/10.1093/aesa/77.2.165

Winkler, D.W. & Sheldon, F., H. (1993) Evolution of nest construction in swallows (Hirundinidae): a molecular phylogenetic perspective. *Proceedings of the National Academy of Sciences,* 90, 5705-5707. doi:10.1073/pnas.90.12.5705

Wong, J.W.Y., Meunier, J. & Kölliker, M. (2013) The evolution of parental care in insects: The roles of ecology, life history and the social environment. *Ecological Entomology,* 38, 123-137. https://doi.org/10.1111/een.12000

Yip, E.C. & Rayor, L.S. (2013) The influence of siblings on body condition in a social spider: Is prey sharing cooperation or competition? *Animal Behaviour,* 85, 1161-1168. https://doi.org/10.1016/j.anbehav.2013.03.016

Publications and Manuscripts

Publication 1: Parental feeding and defense of young facilitate faster offspring growth

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Ready to submit

Sa.S. conceived the study; M.A.P, A.C.-N., and Sa.S. designed the experiments. All authors helped to collect beetles. M.A.P., A.C.-N. and M.T. collected the data; M.A.P. analysed the data; A.-K.E, M.A.P., S.K.S., and Sa.S. discussed the results, A.-K.E. and M.A.P. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Abstract

The factors promoting the evolution of parental care remain largely unknown, despite extensive efforts to develop a unifying theory. The unusual degree of interspecific variation in parental care in carrion beetles, from no care at all to the advanced care of burying beetles (*Nicrophorus*), enabled us to test a key prediction that offspring in species with more elaborate care should exhibit faster growth. We conducted a phylogenetically controlled, common-garden study of 17 species across the full spectrum of parental care, measuring larval growth rates under two conditions: i) in the absence of parental care (all species) and ii) in the presence of parents (parental species only). When reared under natural conditions, larval growth rates were strongly affected by the level of parental care; *Nicrophorus* larvae grew the fastest, followed by *Ptomascopus morio*, a species that guards its young but does not feed them, and then by the non-parental Silphini. Our results support the hypothesis that the faster larval growth of offspring in parental species was an adaptive consequence of 1) parental provisioning, permitting larvae to allocate more resources towards their own growth, and 2) parental defence, which emancipated larvae from the need to invest in their own antipredator adaptations.

Introduction

The ontogenesis of multicellular organisms entails both growth (increase in biomass) and development (increase in specialized tissue and information content). Similar growth patterns in diverse organisms prompted the first attempts to find generalized equations that describe them (Pütter 1920; von Bertalanffy 1938). Although such mechanistic models have since been greatly improved and refined (see Ricklefs 2003; Moses *et al.* 2008; Kooijman *et al.* 2020; Sibly & Brown 2020), they all require information about specific parameters to estimate the increase in resource acquisition (feeding and digestion) and use (maintenance and supplying energy to tissues) during an organism's growth. The trajectory of growth reflects the balance between inputs and outputs of energy and material resources (Sibly & Brown 2020); growth slows with increasing size and eventually ceases because resource use rises faster than its acquisition. These models emphasize constraints on supply and demand, while other aspects of an organism’s energy balance, such as reproduction or immunity, are largely disregarded (Marshall & White 2019).

Life-history (LH) theory, in contrast, approaches growth patterns from an ultimate perspective. It posits that organisms can only maximize their lifetime fitness if they optimally balance their allocation of energy into the competing biological demands of growth, maintenance, and reproduction within a specific environmental context (e.g., Stearns 1992). Variation in growth and resource allocation is assumed to result from different selective pressures under different biotic and abiotic conditions. LH theory predicts maximization of early growth rates because juvenile mortality is higher than adult mortality and under strong selection because of its outsize effects on lifetime fitness (Roff 2000). The physiological processes involved in the acquisition, processing, and allocation of resources are mostly disregarded and viewed as an outcome of selection rather than as an important constraint.

Attempts to integrate these two perspectives are rare, although ultimate and proximate factors shape most traits. Food availability, foraging behaviour, and digestive morphology and physiology affect the acquisition and assimilation of resources, while the optimal allocation of resources by growing juveniles to the competing demands of growth, maintenance (basic metabolism plus defence), storage, foraging, and reproduction will likely depend on the relative importance of these functions for their survival and reproduction (Boggs 2009).

Resource allocation patterns of juveniles can be affected by their social environment. Parents caring for developing juveniles can protect young from environmental hazards (predators, pathogens, desiccation, flooding, etc.) and facilitate food intake by provisioning or direct feeding (Wilson 1975; Clutton-Brock 1991; Smiseth, Kölliker & Royle 2012). Such care can benefit both parents and young via the increased fitness of current offspring (Hamilton 1964b; Wilson 1975; Clutton-Brock 1991; Klug, Alonzo & Bonsall 2012). With parents providing protection and food, the young need fewer of their own resources for defensive and foraging adaptations. Consequently, juveniles receiving parental care should be able to assimilate more resources and reduce allocation to maintenance functions, thus permitting faster growth and development.

The general conditions favouring the initial evolution of parental care in diverse taxa have remained largely enigmatic, despite decades of attempts to find a unifying theory for the evolution of care (see Royle, Smiseth & Kölliker 2012; Kramer & Meunier 2019). Accelerated growth can increase survival by abbreviating risky life-history stages, an important benefit of care even in its incipient stages (Wilson 1975; Clutton-Brock 1991; Klug & Bonsall 2009; Klug, Alonzo & Bonsall 2012; Klug & Bonsall 2014). Although comparative studies could help elucidate the relationship between parental care and growth rates or development times, the lack of interspecific variation in the extent of care in most taxa hinders a comparative approach. In altricial birds and eutherian mammals, for example, care is invariably required for young to survive (Clutton-Brock 1991; Royle, Smiseth & Kölliker 2012).

However, taxa with more diverse parental care exist. We chose carrion beetles (Coleoptera: Staphylinidae: Silphinae) for our study of juvenile growth rates and their association with parental care. This taxon was previously considered a family ('Silphidae'), but recent molecular phylogenetic analyses have led to its reclassification within the Staphylinidae (Cai *et al.* 2022; Růžička *et al.* 2023). It contains species without parental care, species that only defend the young, and species that defend and feed them. All the species in our study reproduce on carrion. This rare degree of interspecific variation within a limited taxonomic group exploiting a similar resource enabled us to test the general prediction that growth rates should be higher in species with more extensive parental care because parental regurgitation allows larvae to acquire more resources, and parental defence reduces their need to invest in their own defence.

Carrion is considered a 'bonanza' resource (protein-rich but scarce and ephemeral, Wilson 1975), with intense competition between its users. Many insects with necrophagous larvae, especially larger flies and beetles, have evolved early carrion detection, high reproductive rates, and rapid larval development as adaptations to this food source (Evans, Wallman & Barton 2020). However, only burying beetles (*Nicrophorus*) are able to bury small carcasses as the sole food source for the young. They prepare, maintain, and defend the buried carcass, regurgitate predigested food to larvae and defend them from predators (Pukowski 1933). In their sister genus *Ptomascopus*, where only one (*P. morio*) of three species (Sikes, Madge & Newton 2002) has been studied, females defend the young from predatory rove beetles (Trumbo, Kon & Sikes 2001; Suzuki & Nagano 2006), but they do not feed larvae nor bury carcasses (Peck 1982; Trumbo, Kon & Sikes 2001; Suzuki & Nagano 2006). Due to the direct feeding of young by adults, we expect larvae to grow faster when parental adults are present in all *Nicrophorus spp.* but not in *P. morio*.

In addition to parental care, our study species also vary in adult, egg, and larval size. In *Nicrophorus*, differences in larval growth rate might account for species differences in adult size (Trumbo 1992). If larger species attain larger adult size by growing faster rather than by growing longer, adult size and juvenile growth rate should covary across species. If species with especially large eggs have lower growth rates early in larval development because their hatchling larvae are already quite large, we should see a negative correlation between egg size and growth rate in the Silphini and in the genus *Nicrophorus*.

All the Silphini in our study reproduce on carrion without providing care, with females ovipositing near a carcass and leaving shortly thereafter. Their larvae must move around the carcass to forage or hide, and they have costly morphological and behavioural defensive adaptations including heavier sclerotization and greater agility (Dorsey 1940; Anderson 1982, see **Fig. S1**). This means that the Silphinae as a whole exhibit at least three distinctly different levels of parental care: (1) no care in members of the tribe Silphini (*Thanatophilus sinuatus*, *Necrophila americana*, and *Oiceoptoma thoracica*), (2) defence against predators and reduction of competition in *P. morio*, and (3) parental resource modification and concealment, protection from predation and competition, and direct regurgitation in *Nicrophorus.* We predict that these different levels of care should result in different growth rates. Under experimental conditions that mirror the care state of natural broods (with parental adults present in *Ptomascopus* and *Nicrophorus* but absent in the other genera), growth rates should be highest in *Nicrophorus*, intermediate in *Ptomascopus*, and lowest in the Silphini. When we experimentally exclude parents, we predict different results. The larvae of both *Nicrophorus* and *Ptomascopus* have evolved to grow up under the protection of parental adults and do not have to invest in costly antipredator adaptations such as body armour. Even without parents, they should be able to grow faster than larvae of the Silphini, except in those species that require parental feeding to survive (Trumbo 1992; Capodeanu-Nägler *et al.* 2016) and two such species are included in our study.

Methods

Origin and husbandry of beetles

This study includes 17 species of Silphinae, 14 in the Nicrophorini and three in the Silphini (sensu Sikes & Venables 2013; Cai *et al.* 2022; Růžička *et al.* 2023). *Ne. americana, N. interruptus, N. investigator, N. nepalensis, N. tenuipes, N. tomentosus, O. thoracica, P. morio,* and *T. sinuatus* individuals were field-caught. All other beetles came from outbred laboratory populations established with field-caught beetles (for geographic origin and the number of generations in the laboratory, see **Table S1.1**). All beetles were kept at 20 °C on a 16:8 h light:dark cycle and fed cut-up mealworms (*Tenebrio molitor* and *Zophoba morio*) *ad libitum* twice weekly, but *N. americanus* adults were fed chicken liver.

Experimental design and procedures

To induce reproduction, non-sibling pairs of beetles were provided with a thawed previously frozen carcass in plastic containers two-thirds filled with moist peat (*N. americanus*: 29 × 18 × 11 cm, all others: 10 x 10 x 6 cm). We chose 20-g mouse carcasses (range: 17.5-22.5 g) as the standard size because most species reproduce successfully on this carcass size.

N. americanus require larger carcasses (Kozol, Scott & Traniello 1988) and were provided with 100-g rats instead (range: 97.5–110 g). A general problem with such common garden-type interspecific comparisons is that the standardized conditions cannot be optimal for all tested species, but our conditions proved acceptable to the species in the study. All *Nicrophorus* buried carcasses and oviposited in the substrate, and the other species in our study laid eggs near unburied carcasses. The day before larvae were expected to hatch, we transferred each pair and its carcass to a new container with peat to reliably separate the larvae from their parents. The eggs were gently collected from the soil with flexible forceps or small brushes. We photographed at least ten eggs from each of 15 broods for each species using a binocular microscope (Stemi 2000-CS, magnification 6.5 – 50x, ZEISS, Oberkochen, Germany) connected to a camera (Canon EOS 500D, Tokyo, Japan) to measure the width (w) and length (L) of the eggs using ImageJ. For broods with fewer than ten eggs, we photographed all available eggs. In Nicrophorini, eggs are ellipsoid-shaped, and we calculated volume as $V = \frac{1}{6}\pi w^2 L$ (Berrigan 1991); for the spherical eggs of the Silphini, we used the formula $V = \frac{1}{6}\pi\left(\frac{w+L}{2}\right)^3$. We returned eggs to the boxes they had come from and checked at least every 8 h for hatched larvae. To facilitate growth measurements without having to mark individual larvae, we added 15 larvae to each carcass simultaneously, combining unrelated larvae hatching at similar times. In *T. sinuatus*, we had to use fewer larvae (1-5) because we caught the beetles at the end of their reproductive season and many failed to reproduce.

The larvae of the non-parental Silphini were placed on a mouse carcass where a pair had produced eggs, but without adults ('unattended'). For all Nicrophorini, we had two treatment groups; larvae were placed on a carcass prepared by parental beetles, either with those beetles ('full parental attendance') or without parents ('pre-hatching attendance').

Although *P. morio* do not regurgitate to their offspring, parental presence can be beneficial for larvae that are faced with predatory rove beetles or competing carrion fly larvae on the carcass (Trumbo, Kon & Sikes 2001; Suzuki & Nagano 2006). Therefore, we treated *P. morio* like the *Nicrophorus* species and assessed their growth rates under conditions of either full or pre-hatching parental attendance. When larvae were placed with parents, we only used parents whose own larvae had already hatched to avoid the time-dependent infanticide of early larvae exhibited by *Nicrophorus* (Müller & Eggert 1990). *Nicrophorus* parents chew a distinct hole into the top of the carcass before or shortly after the first larvae appear on the carcass (Pukowski 1933), which facilitates larval access to the inside of the carcass (Eggert, Reinking & Müller 1998). Therefore, we cut a hole into carcasses that did not have one before adding the 15 larvae. To standardize conditions across species, we did the same for carcasses provided to Silphini. Larvae of all species were weighed using a precision scale (Kern ABJ 120-4M, Kern und Sohn GmbH, Balingen, Germany) before being placed on a carcass (0 h), and surviving larvae were weighed again after 48 h and at the time of dispersal when they left the remains of the carcass (see **Table S1.2**). We calculated early relative growth rates of larvae as $GR = \frac{lm_{48} - lm_0}{lm_0}$, where lm_0 and lm_{48} are the average larval masses in a brood at hatching and at 48 h, respectively (e.g., Prang *et al.* 2022). We chose to focus on early growth in the first 48 h rather than assessing the overall rate of growth on the carcass because the benefit of parental regurgitations appears to be greatest in very young larvae (Pukowski 1933; Eggert, Reinking & Müller 1998; Smiseth, Darwell & Moore 2003). Moreover, the exact time of dispersal can be difficult to assess objectively, both in species without care where developing larvae mostly remain hidden under the carcass and in *Nicrophorus*, where dispersal time can vary even within broods. In some *Nicrophorus* species, few larvae survive without care (Trumbo 1992). If there were unattended survivors, we analysed their actual growth rate; if there were none, we ascribed a growth rate of zero to unattended larvae but did not use these values for statistical comparisons (see **Table S1.3** for sample sizes).

Statistics

All data were processed, analysed and, plotted using R (version 4.2.2, R Core Team (2021)) loaded with the packages: 'ape_5.6-2', 'caper_1.0.1', 'car_3.1-1', 'castor_1.7.6', 'cowplot_1.1.1', 'emmeans_1.8.4-1', 'ggplot2_3.4.0', 'ggpmisc_0.5.2', 'ggpubr_0.5.0', 'ggrepel_0.9.3', 'ggtree_3.4.0', 'multcomp_1.4-20', and 'phylobase_0.8.10'.

Testing for correlated evolution of growth with egg size or body size

We first tested if larval growth rates correlate with adult body size under typical care conditions to test the idea that larger species grow faster. In contrast to many other studies of *Nicrophorus*, we did not use adult pronotum width as a proxy for body size, because the pronotum and body shape of the Silphini differ a lot from those of the Nicrophorini. We used the dispersal mass of larvae reared under their species-typical care regimen instead because it can be compared between tribes and is closely correlated with adult size in *Nicrophorus* (Bartlett & Ashworth 1988; Trumbo & Xhihani 2015). Dispersal mass values were log-transformed because of the skewed distribution of untransformed values.

We tested for correlated evolution of growth rate with larval dispersal mass or egg size (egg volume). Because taxon-specific differences occur and the overall analysis should not be biased towards more speciose groups, such analyses should always correct for phylogenetic effects. Such a correction can be accomplished using phylogenetically independent contrasts (Felsenstein 1985) or phylogenetic least squares functions that require information about phylogenetic distances. These were only available for the Nicrophorini (Sikes & Venables 2013, see **Fig. 1.1A**), although there is a reliable phylogenetic tree for all tested species (Dobler & Müller 2000; Sikes & Venables 2013). Therefore, we calculated phylogenetic least squares for the Nicrophorini only, using the function pgls() of the ‘caper’ package in R to fit a model assuming trait evolution under Brownian motion only. For the calculation of phylogenetically independent contrasts (PIC) in analyses involving all Silphinae, we used the function pic() of the ‘ape’ package in R, which calculates PIC after Felsenstein (1985) to test for evidence that the variables in question evolve in concert. We used a branch length transformation after Grafen (1989) and calculated the maximum likelihood for the PICs assuming trait evolution under Brownian motion.

The effect of parental presence in species with parental care

We tested whether post-hatch care affects early larval growth rates in the Nicrophorini with a linear model (LM) examining the effects of species, care type, and their interaction on larval growth rate at 48 h, followed by pairwise comparisons with adjusted alpha levels using the Holm sequential method (Holm 1979) for multiple testing within each species. This comparison excluded the species that do not survive without parents (*N. americanus* and *N. orbicollis*).

Comparing growth in species with different levels of parental care

Finally, we tested whether different levels of care were associated with different growth rates, both under care conditions similar to natural broods (with parental adults present in Nicrophorini but absent in Silphini), and without post-hatching care. We had predicted significant differences in growth between *Nicrophorus*, *Ptomascopus*, and the Silphini under natural conditions, and differences only between Nicrophorini and Silphini without care (see Introduction). We used two separate LMs to test these predictions, followed by pairwise comparisons with a Holm correction (Holm 1979).

Results

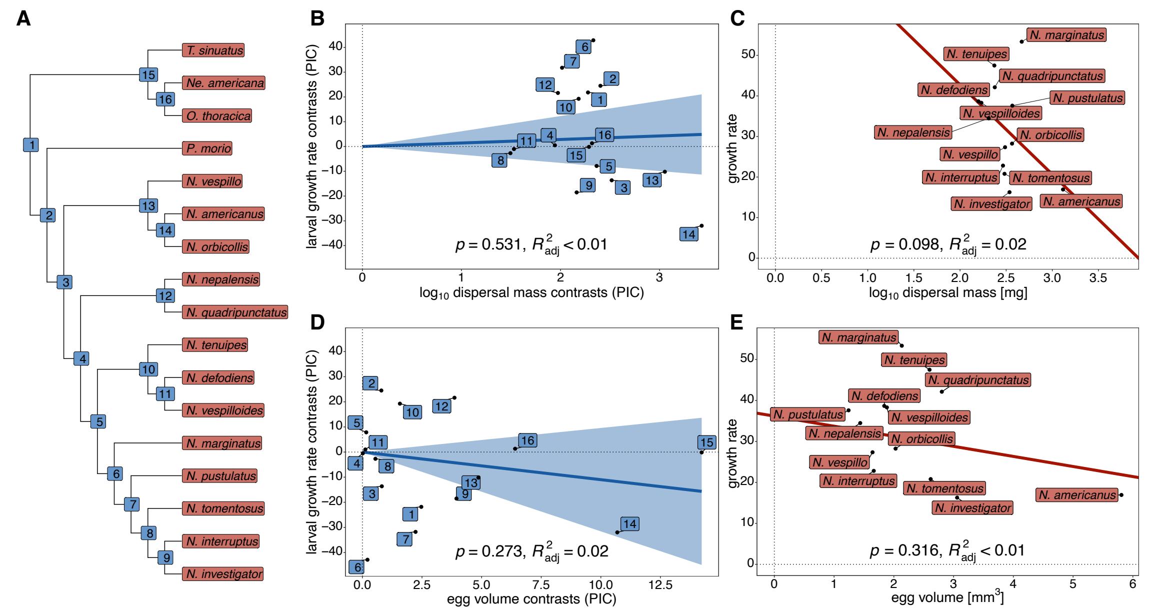

Figure 1.1

(A) The phylogeny of the tested Silphinae after Dobler and Müller (2000) and Sikes and Venables (2013); growth rate as a function of dispersal mass using phylogenetically independent contrasts (PICs) for all Silphinae (B) and PGLS for *Nicrophorus* (C); and growth rate as a function of egg volume using PICs for all Silphinae (D) and PGLS for *Nicrophorus* (E). Data points (blue) in (B) and (D) indicate the contrasts at the nodes shown in (A), and the shaded regions show the 95 % confidence intervals. None of the resultant relationships were significant. (© Figure adapted from Prang, Eggert *et al.* (in preparation))

Correlated evolution of growth with egg size or body size

We found no evidence for a correlated evolution of growth rate and adult size (log transformed dispersal mass), neither in the Silphinae (PICs with a Grafen branch length transformation, LM: $F_{1,14} = 173.4$, $p = 0.53$, $R^2_{adj} < 0.01$, **Fig. 1.1B**) nor within the genus *Nicrophorus* (PGLS: F = 3.27, p = 0.098, df = 10, $R^2_{adj} = 0.16$, **Fig. 1.1C**). If larger species did arrive at their larger adult size by growing faster, adult size should have been correlated with growth rate.

We also found no significant relationship between egg size and early growth rate, as revealed in an analysis using phylogenetically independent contrasts for the Silphinae (LM: $F_{1,14} = 516.8$, $p = 0.27$, $R^2_{adj} = 0.018$, **Fig. 1.1D**) and for the genus *Nicrophorus* (PGLS: F = 1.10, p = 0.32, df = 10, $R^2_{adj} < 0.01$, **Fig. 1.1E**). If early growth rates were lower for species with larger eggs, then egg size should have been negatively correlated with growth rate.

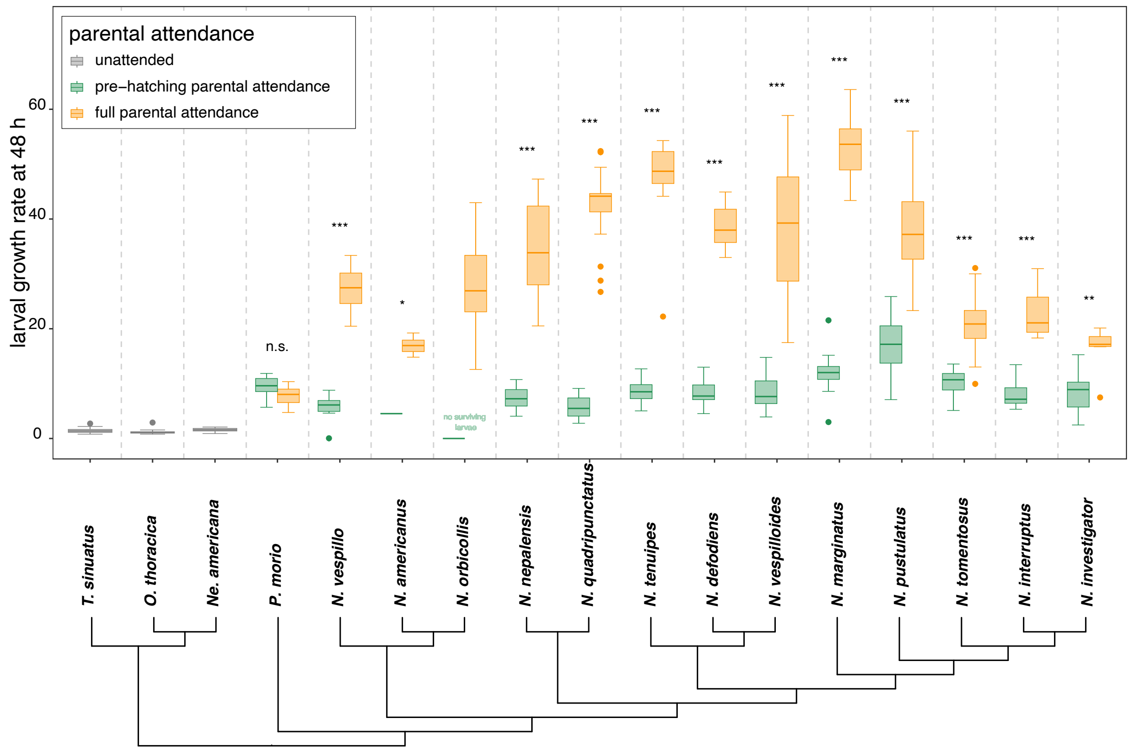

Figure 1.2
Larval growth rates of the tested Silphinae in the first 48 h after hatching, graphed onto a cladogram. Larvae were unattended (grey), or they received pre-hatching (green) or full parental care (orange). Boxplots show median, interquartile range, and range of growth rates for surviving larvae. The dots are values that fall outside the interquartile range (>1.5 × interquartile range). Asterisks indicate statistically significant differences between larvae with pre-hatching attendance and full parental attendance (*: p < 0.05, **: p < 0.005, ***: p < 0.001). Cladogram based on Dobler and Müller (2000) and Sikes and Venables (2013). (© Figure adapted from Prang, Eggert et al. (in preparation))

The effect of parental presence in species with parental care

Our study revealed a significant interaction between parental presence and species in their effect on early growth rates (LM: $F_{13,417} = 34.26$, $p < 0.001$) and significant effects of both parental attendance (LM: $F_{1,429} = 1076.31$, $p < 0.001$) and species (LM: $F_{13,417} = 45.14$, $p < 0.001$). *Nicrophorus* larvae attended by parents grew significantly faster than unattended ones (**Fig. 1.2**; *post hoc* pairwise comparisons with Holm-adjusted *p*-values: p = 0.042 for *N. americanus*, $p = 0.0056$ for *N. investigator*, p < 0.001 for all other *Nicrophorus* species, but no statistics for *N. orbicollis*). Few or no larvae of *N. americanus* and *N. orbicollis* survived to 48 h without parents (**Table S1.2**), but in all other *Nicrophorus*, larval survival without parents was above 10 %. In *P. morio*, larval growth rates did not increase with full parental attendance (**Fig. 1.2**; *post hoc* pairwise comparison with Holm-adjusted *p*-value: $p = 0.42$).

Comparing growth in species with different extent of parental care

Under natural conditions, with parents present in *Ptomascopus* and *Nicrophorus* but absent in the other genera, larval growth rate was affected by the extent of parental care (no care vs. defence only vs. feeding and defence, LM: $F_{2,325} = 306.42$, $p < 0.001$). *Nicrophorus* larvae grew the fastest, faster than both *Ptomascopus* (**Fig. 1.2**; *post hoc* comparison with Holm-adjusted *p*-values: $p < 0.001$) and the Silphini (**Fig. 1.2**; $p < 0.001$). *Ptomascopus* larvae grew faster than the Silphini (**Fig. 1.2**; $p = 0.029$).

Comparing growth rates in the absence of parents, we found that the level of care still influenced larval growth (**Fig. 1.2**; LM: $F_{2,265} = 83.92$, $p < 0.001$). The larvae of Silphini whose adults never provide parental care grew more slowly than unattended *P. morio* larvae (**Fig. 1.2**; *post hoc* comparison with Holm-adjusted *p*-value: $p < 0.001$), and more slowly than burying beetle (*Nicrophorus*) larvae (**Fig. 1.2**; *post hoc* comparison with Holm-adjusted *p*-value: $p < 0.001$). Unattended *Ptomascopus* and *Nicrophorus* larvae did not differ in their larval growth rate (**Fig. 1.2**; *post hoc* comparison with Holm-adjusted *p*-value: $p = 0.39$).

Discussion

Our analysis of growth rates in carrion beetles supports the hypothesis that parental care allows developing larvae to allocate more resources towards their own rapid growth. We had predicted that growth rates would increase with more complex and elaborate parental care, with both parental feeding and protection contributing to this effect. When we tested this under conditions emulating the natural situation (with parents in Nicrophorini, without in the Silphini), early growth rates were highest in *Nicrophorus* (feeding and protection), intermediate in *P. morio* (protection only), and lowest in the Silphini (no parental care). However, we found no evidence of correlated evolution between larval growth rate and adult size, or growth rate and egg size. Growth rates did not depend on egg or hatchling size, neither within all Silphinae nor among *Nicrophorus* species. They were also not correlated with the size of larvae dispersing from the carcass, refuting the suggestion that larger species grow faster.

We documented accelerated growth in species that guard their young and even faster growth in species that both guard and feed them. Although both components of parental care afford the larvae the opportunity for faster growth, we see these as different effects. We think that accelerated growth in the Nicrophorini (*Nicrophorus* and *Ptomascopus*) evolved as a result of their shared derived trait of parental guarding that allowed for reduced defensive adaptations in the larvae. Female

Nicrophorini protect their larvae from predators by attacking and even killing potential predators like rove beetles (Pukowski 1933; Scott 1990; Suzuki & Nagano 2006). The unprotected larvae of the Silphini, in contrast, invest in costly antipredator adaptations such as body armour, defensive secretions, and neural structures mediating defensive behaviour and rapid movement. Melanization and sclerotization are costly features (Talloen, Van Dyck & Lens 2004; Andersen 2010; Maddrell 2018), and locomotion carries an obvious cost through muscular activity. Even the original descriptions of the larvae of Silphini highlighted their sclerotization and quick, agile movements (e.g., von Lengerken 1938; Dorsey 1940; Ratcliffe 1972; Ratcliffe 1996). In nicrophorine larvae, these defensive traits are absent, and their diminished agility, more cylindrical body shape, and minimal sclerotization and melanization are used as a distinguishing feature in dichotomous keys (Anderson 1982; Anderson & Peck 1985). The taxonomic division into the tribes Silphini and Nicrophorini (formerly subfamilies Silphinae and Nicrophorinae) is supported by numerous molecular phylogenies (Dobler & Müller 2000; Ikeda *et al.* 2008; Sikes & Venables 2013; King *et al.* 2015) and unaffected by the recent re-classification of the former family Silphidae (Staphylinidae; Cai *et al.* 2022; Růžička *et al.* 2023). The divergent larval traits in these tribes are now invariant within species and unaffected by conditions during larval development, resulting in higher growth rates in larvae of the Nicrophorini even when they are not attended and defended.

The faster growth with parental feeding that we observed in *Nicrophorus* (**Fig. 2**) is, however, largely a proximate effect. Like other silphine larvae, *Nicrophorus* larvae can self-feed, but larvae with feeding adults always grow faster than unattended conspecifics (**Fig. 2**, Eggert, Reinking and Müller (1998); Rauter and Moore (2002); Smiseth, Darwell and Moore (2003); Smiseth, Lennox and Moore (2007). This intraspecific increase in growth rate in *Nicrophorus* larvae with parental attendance reflects the greater resources assimilated by fed larvae. To our knowledge*, Nicrophorus* species are the only Silphinae in which adults directly regurgitate to the larvae (Pukowski 1933; Sikes & Venables 2013). In their close relative *P. morio*, where larvae do not interact with adults (Peck 1982; Trumbo, Kon & Sikes 2001; Suzuki & Nagano 2006), the growth rate was unaffected by parental presence. Although current feeding effects in *Nicrophorus* may be proximate, the evolution of parental feeding must have also involved permanent changes in larval physiology as a result of parent-offspring coadaptation. Only such coevolution can explain the extreme phenotypic plasticity of larval growth rates (**Fig. 2**) and the complete dependence on parental feeding in some species (all unattended *N. orbicollis* and most unattended *N. americanus* larvae died soon after hatching).

The measures taken for larval protection in *Nicrophorus* are far more sophisticated than in *Ptomascopus*. Although only *Nicrophorus* parents conceal the carcass from predators and competitors (Suzuki 1999; Trumbo & Sikes 2021) and hinder decay using antimicrobial substances (Arce *et al.* 2012), growth rates in unattended nicrophorine larvae are similar. Faster growth in unattended *Nicrophorus* may be impossible due to food intake constraints, or it may simply not have evolved if broods in the field are rarely ever unattended. The most sophisticated parental care behaviours, such as kin discrimination and larval infanticide (Müller & Eggert 1990; Eggert & Müller 2000; Eggert, Otte & Müller 2008; Eggert & Müller 2011), are not likely to affect larval growth and probably evolved in the context of avoiding misdirected care and optimizing resource use.

Growth rate, egg size, and parental care

In the species we studied, egg size was unrelated to growth rate. *Ne. americana* and *N. americanus* produce the largest eggs but have low growth rates. However, there was no significant negative correlation between egg size and growth either, neither in the Silphinae nor in the genus *Nicrophorus* (**Fig. 1.1D, Fig. 1.1E**). Most *Nicrophorus* species have very small eggs, possibly due to the fact that their breeding resource, small carrion, requires quick oviposition because it degrades faster than larger carcasses. *Nicrophorus* egg production is further constrained by the fact that females can only oviposit hours or even days after they find and evaluate a carcass (Scott & Traniello 1987; Trumbo & Robinson 2004). Faster production and development of small eggs (García-Barros 2000; Maino *et al.* 2016) allows the larvae to begin consuming the carcass sooner. Laying more eggs can be advantageous, especially in competitive situations (Müller & Eggert 1990; Eggert & Müller 2000; Eggert, Otte & Müller 2008; Eggert & Müller 2011), and the need to produce many eggs quickly may limit their individual size. In *N. pustulatus*, which can produce unusually large clutches (Trumbo 1992), eggs are especially small (**Table S1.2**), suggesting a size/number trade-off. Females use material from the breeding resource itself (Eggert, Otte & Müller 2008) and from food consumed before arriving on a carcass (Trumbo & Robinson 2008) for egg production.

Although larger females lay larger eggs and rear larger offspring, offspring size only depends on the size of the caring female, not the size of the egg (Steiger 2013). When parents provide care, egg size does not affect the final body size of offspring (Monteith, Andrews & Smiseth 2012). Similarly, variation in egg size is usually irrelevant for offspring survival and size at independence in birds (Ricklefs 1984). The effects of parental feeding dwarf any selective advantage for larger eggs, which may also explain why altricial bird species with extensive parental care often have smaller eggs than precocial ones (Wesołowski 1994; Williams 1994; Starck & Ricklefs 1998).

Data on egg size and growth rates in insects are scarce because their growth is greatly affected by temperature and other environmental characteristics. Inter- and intraspecific variation in egg size and shape can be affected by oviposition substrates (Church *et al.* 2019), day length, temperature, and female nutrition (Chown & Gaston 2010) or food preference (Ikeda *et al.* 2008), complicating the detection of possible interspecific patterns. We do not know of any large-scale analyses of insect growth rates in relation to egg size. Maino *et al.* (2016) found longer embryonic development times for species with larger eggs. In butterflies, species with larger eggs tend to have longer embryonic and larval development periods (García-Barros 2000), but this could be due to slower growth rates, larger final sizes, or both.

Parental care and egg size might also coevolve. Gilbert and Manica (2010) suggested that insect parental care should be associated with larger egg sizes and smaller egg numbers, but in their analysis, egg size covaried only with the body size of adult females. Frogs with parental care often have large eggs, but the association likely started with large eggs, not parental care (Summers, McKeon & Heying 2006); in our sample, there clearly was no such correlation between egg size and care.

Growth and body size

Burying beetles are the only Silphinae in which body size is under strong positive selection due to intense contest-type competition (Pukowski 1933; Bartlett & Ashworth 1988; Otronen 1988; Müller, Eggert & Dressel 1990; Trumbo 1990; Müller *et al.* 2007). Parental feeding allows larvae to grow faster and reach a larger size such that they will become more competitive adults (Eggert, Reinking & Müller 1998; Satou, Nisimura & Numata 2001; Rauter & Moore 2002; Smiseth, Lennox & Moore 2007). However, our study showed that between species, larger size is not always associated with faster growth and greater dependency on care as (echoed by Jarrett *et al.* 2017) has been suggested. Our largest species (*N. americanus*) was very dependent on care (**Table S1.2**) but had one of the slowest growth rates (**Fig. 1.2**). Larger species can attain their larger size by growing faster, but also by growing for longer periods. *N. orbicollis* did not grow faster than *N. vespilloides* (**Fig. 1.2**), but Benowitz, Moody and Moore (2015) found that it had a longer growth period.

Positive correlations between adult size and juvenile growth rate have been documented in vertebrates (Case 1978; Cooney *et al.* 2020), but even there, body size may explain less variation in growth rates than previously thought. In mammals and birds, larger individuals have lower metabolic rates that in turn are associated with slower growth rates (von Bertalanffy 1938; Peters & Wassenberg 1983; Robinson, Peters & Zimmermann 1983; West, Brown & Enquist 2001), and extrinsic ecological factors such as predation risk and latitude can have strong effects on growth rates (Remeš, Matysioková & Vrána 2020). In insects, no simple relationship between adult size and growth rate has been documented. In addition to the issues with growth rate detailed in the previous section, adult body size is also affected by many proximate and ultimate factors (Chown & Gaston 2010) further precluding reliable comparative studies of growth rate and size.

Evolution of elaborate parental care and rapid growth in *Nicrophorus*

We contend that larval development could become accelerated under parental care, facilitated by the defence, and feeding of young. The very elaborate care of extant *Nicrophorus* could only evolve once parents were able to find and sequester small carcasses. Adults hide carcasses under soil or plant litter, defend them, prepare them by rounding them and removing fur or feathers, oviposit off the carcass and wait until eggs hatch. They feed and defend the larvae while also defending the carcass itself (Pukowski 1933; Eggert & Müller 1997; Scott 1998; Trumbo & Valletta 2007). Eggert and Müller (1997) proposed that burying small carcasses was likely a critical early step in the evolution of care that reduced the number of intra- and interspecific competitors attracted to carcasses, as documented by field studies in the US and Japan (Suzuki 1999; Suzuki 2000; Trumbo & Sikes 2021). Very low-cost parental behaviours like eating fly maggots or attacking insect predators (Scott 1990) could have evolved on unburied carcasses, but the high probability of losing the young or the carcass to predators and scavengers would have negated benefits of more costly care behaviours. Even the above low-cost care may become easier and more beneficial on a concealed and isolated carrion ball, where the risk of caring for unrelated young is also minimized, favouring the continuing evolution of more elaborate care. The use of small carcasses that decay more rapidly likely selected for faster growth and more efficient carcass-to-insect conversion of biomass. Although some Silphini can occasionally be found on small carcasses, these species have highly active predatory larvae that can survive even after the carcass is gone.

With fewer competitors on buried carcasses, fights were a cost-effective way to exclude smaller congeners. Measures that improved the retention of the carcass and maintained its quality were favoured, such as shaping it into a ball, constructing a crypt with coherent walls around it (Pukowski 1933), producing and applying chemicals that control microbial volatiles (Trumbo &

Steiger 2020), closing feeding holes (Trumbo *et al.* 2021), moistening the surface to prevent desiccation (Pukowski 1933), and controlling fungal growth (Suzuki 2001; Hwang & Lin 2013).

Carcass opening and parental regurgitations were likely later steps, possibly precipitated by compacting carcasses into a tight ball covered in intact skin whose interior was harder for larvae to access, process, and digest than the semi-liquid mass of large carcasses in active decay. Parental feeding and larval begging behaviour could then coevolve to become more elaborate (Gardner & Smiseth 2011), leading to greater dependence of larvae on care in some species. Regurgitations increased growth rate and adult size, reduced susceptibility to takeovers, and permitted a more efficient conversion of carrion into larval body mass (Eggert & Müller 1997; Eggert, Reinking & Müller 1998; Trumbo 2016). Frequent interactions between feeding parents and their larvae facilitated the sophisticated fine-tuning of care to current conditions (carcass size, hatching success, presence of conspecifics) and disruptions (presence of intruders, takeovers, hatching failure, or infanticide) that we see today.

Our observations of growth rates in Silphinae are consistent with the hypothesis that they are affected by the respective level of parental care in a species, and the interpretation that both the guarding and the feeding component of care can contribute to increased juvenile growth rates. Although the idea that parental care can affect the speed of development is not novel (e.g., Klug, Alonzo & Bonsall 2012), the argument that not just parental feeding, but also parental protection may facilitate faster juvenile development has not been widely considered in discussions of parental care evolution.

Supplementary Material

Figure S1.1
Larva of *Necrophila americana* (top). *Ne. americana* larvae are characterized by a sclerotized, dark, and opaque cuticle. 3rd instar larva of *Nicrophorus* ssp. (bottom). Larvae of the genus *Nicrophorus* are characterized by a soft, weakly-sclerotized, light-coloured cuticle. (© Figure adapted from Prang, Eggert et al. (in preparation))

Table S1.1

Species and original collection sites of experimental beetles included in the study.

Subfamily	Species	wild-caught or laboratory-bred	Original collection site
Nicrophorini	*N. americanus*	laboratory-bred (2nd gen.)	USA, OK, Cherokee Wildlife Management Area (CWMA) in Cherokee (35°43'05.15’ N, 95°04'23.88’W)
Nicrophorini	*N. defodiens*	laboratory-bred (4th gen.)	USA, CT, Colebrook (42°00'00.0’N 73°04'48.0’W)
Nicrophorini	*N. interruptus*	wild-caught	Germany, Alsbach and Lampertheim (49°44'28.64’N, 08°38'00.48’E) (49°36'13.77’N, 08°32'35.37’E)
Nicrophorini	*N. investigator*	wild-caught	USA, AK, Fairbanks (64°54'04.7’N 147°31'43.0’W)
Nicrophorini	*N. marginatus*	laboratory-bred (3rd gen.)	USA, UT, Goshen (40°12'46.5’N, 111°48'09.0’W)
Nicrophorini	*N. nepalensis*	wild-caught	Taiwan, Alishan (23°30'30.6’N, 120°48'00.9’E)
Nicrophorini	*N. orbicollis*	laboratory-bred (8th gen.)	USA, IL, Lexington (40°39'57’N, 88°53'49’W)
Nicrophorini	*N. pustulatus*	laboratory-bred (3rd gen.)	USA, IL, Lexington (40°39'57’N, 88°53'49’W)
Nicrophorini	*N. quadripunctatus*	laboratory-bred (3rd gen.)	Japan, Niigata, Nagaoka (37°28'01.2’N 138°43'51.6’E)
Nicrophorini	*N. tenuipes*	wild-caught	Shibecha, Hokkaido, Japan (43°11'24.0’N 144°22'12.0’E)
Nicrophorini	*N. tomentosus*	wild-caught	USA, IL, Lexington (40°39'57’N, 88°53'49’W)
Nicrophorini	*N. vespillo*	laboratory-bred (5th gen.)	Germany, Freiburg (48°02′14″N, 07°50′52″E)
Nicrophorini	*N. vespilloides*	laboratory-bred (11th gen.)	Germany, Ulm (48°25'03’N, 09°57'45’E)
Nicrophorini	*P. morio*	wild-caught	Japan, Chiba, Sambu City (35°37'12.0’N 140°21'00.0’E)
Silphini	*Ne. americana*	wild-caught	USA, IL, Lexington (40°39'57’N, 88°53'49’W)
Silphini	*O. thoracica*	wild-caught	Germany, Bayreuth (49°55'15.6’ N, 11°34'19.2’ E)
Silphini	*T. sinuatus*	wild-caught	Germany, Alsbach and Lampertheim (49°44'28.64’N, 08°38'00.48’E) (49°36'13.77’N, 08°32'35.37’E)

Table S1.2

Mean values of measured life history traits (egg volume, growth rate and number of surviving broods at 48 h) under different parental attendances (unattended, pre-hatching parental attendance, and full parental attendance).

Subfamily	Species	Average egg volume [mm^3]	Average larval growth rate at 48 h			Number of surviving broods at 48 h		
			Parental attendance			Parental attendance		
			Unattended	Pre-hatching parental attendance	Full parental attendance	Unattended	Pre-hatching parental attendance	Full parental attendance
Nicrophorini	*N. americanus*	5.81	–	4.53	16.93	–	1 of 10 broods	9 of 9 broods
Nicrophorini	*N. defodiens*	1.84	–	7.76	38.67	–	18 of 18 broods	18 of 18 broods
Nicrophorini	*N. interruptus*	1.66	–	8.13	22.82	–	16 of 16 broods	11 of 11 broods
Nicrophorini	*N. investigator*	3.06	–	8.36	16.27	–	13 of 13 broods	6 of 6 broods
Nicrophorini	*N. marginatus*	2.13	–	12.04	53.36	–	18 of 18 broods	17 of 17 broods
Nicrophorini	*N. nepalensis*	1.44	–	7.43	34.48	–	16 of 16 broods	12 of 12 broods
Nicrophorini	*N. orbicollis*	2.03	–	*NA*	28.24	–	0 of 16 broods	35 of 35 broods
Nicrophorini	*N. pustulatus*	1.24	–	16.94	37.59	–	18 of 18 broods	38 of 38 broods
Nicrophorini	*N. quadripunctatus*	2.80	–	5.78	42.13	–	11 of 21 broods	18 of 18 broods
Nicrophorini	*N. tenuipes*	2.60	–	8.56	47.49	–	16 of 16 broods	16 of 16 broods
Nicrophorini	*N. tomentosus*	2.62	–	10.19	20.80	–	15 of 16 broods	17 of 17 broods
Nicrophorini	*N. vespillo*	1.64	–	5.64	27.35	–	9 of 17 broods	16 of 16 broods
Nicrophorini	*N. vespilloides*	1.88	–	8.56	38.31	–	17 of 17 broods	31 of 31 broods
Nicrophorini	*P. morio*	1.57	–	9.49	7.78	–	15 of 15 broods	15 of 15 broods
Silphini	*Ne. americana*	9.02	1.61	–	–	13 of 13 broods	–	–
Silphini	*O. thoracica*	6.75	1.14	–	–	40 of 40 broods	–	–
Silphini	*T. sinuatus*	1.23	1.44	–	–	18 of 18 broods	–	–

References

Andersen, S.O. (2010) Insect cuticular sclerotization: A review. *Insect Biochemistry and Molecular Biology,* 40, 166-178. https://doi.org/10.1016/j.ibmb.2009.10.007

Anderson, R.S. (1982) Burying beetle larvae: Nearctic *Nicrophorus* and Oriental *Ptomascopus morio* (Silphidae). *Systematic Entomology,* 7, 249-264. https://doi.org/10.1111/j.1365-3113.1982.tb00443.x

Anderson, R.S. & Peck, S.B. (1985) The carrion beetles of Canada and Alaska (Coleoptera: Silphidae and Agyrtidae) Part 13. *The insects and arachnids of Canada*. Agriculture Canada, Canadian Government Publishing Center, Ottawa, Canada, ISBN: 0662117525

Arce, A.N., Johnston, P.R., Smiseth, P.T. & Rozen, D.E. (2012) Mechanisms and fitness effects of antibacterial defences in a carrion beetle. *Journal of Evolutionary Biology,* 25, 930-937. https://doi.org/10.1111/j.1420-9101.2012.02486.x

Bartlett, J. & Ashworth, C.M. (1988) Brood size and fitness in *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Behavioral Ecology and Sociobiology,* 22, 429-434. https://doi.org/10.1007/BF00294981

Benowitz, K.M., Moody, K.J. & Moore, A.J. (2015) Are species differences in maternal effects arising from maternal care adaptive? *Journal of Evolutionary Biology,* 28, 503-509. https://doi.org/10.1111/jeb.12573

Berrigan, D. (1991) The allometry of egg size and number in insects. *Oikos,* 60, 313-321. https://doi.org/10.2307/3545073

Boggs, C.L. (2009) Understanding insect life histories and senescence through a resource allocation lens. *Functional Ecology,* 23, 27-37. https://doi.org/10.1111/j.1365-2435.2009.01527.x

Cai, C., Tihelka, E., Giacomelli, M., Lawrence, J.F., Ślipiński, A., Kundrata, R., Yamamoto, S., Thayer, M.K., Newton, A.F. & Leschen, R.A. (2022) Integrated phylogenomics and fossil data illuminate the evolution of beetles. *Royal Society Open Science,* 9, 211771. https://doi.org/10.1098/rsos.211771

Capodeanu-Nägler, A., Keppner, E.M., Vogel, H., Ayasse, M., Eggert, A.-K., Sakaluk, S.K. & Steiger, S. (2016) From facultative to obligatory parental care: Interspecific variation in offspring dependency on post-hatching care in burying beetles. *Scientific Reports,* 6, 29323. https://doi.org/10.1038/srep29323

Case, T.J. (1978) On the evolution and adaptive significance of postnatal growth rates in the terrestrial vertebrates. *The quarterly review of biology,* 53, 243-282. https://doi.org/10.1086/410622

Chown, S.L. & Gaston, K.J. (2010) Body size variation in insects: A macroecological perspective. *Biological Reviews,* 85, 139-169. https://doi.org/10.1111/j.1469-185X.2009.00097.x

Church, S.H., Donoughe, S., de Medeiros, B.A.S. & Extavour, C.G. (2019) Insect egg size and shape evolve with ecology but not developmental rate. *Nature,* 571, 58-62. https://doi.org/10.1038/s41586-019-1302-4

Clutton-Brock, T.H. (1991) *The evolution of parental care*. Princeton University Press, Princeton, New Jersey, USA, https://doi.org/10.1046/j.1420-9101.1992.5040719.x

Cooney, C.R., Sheard, C., Clark, A.D., Healy, S.D., Liker, A., Street, S.E., Troisi, C.A., Thomas, G.H., Székely, T., Hemmings, N. & Wright, A.E. (2020) Ecology and allometry predict the evolution of avian developmental durations. *Nature Communications,* 11, 2383. https://doi.org/10.1038/s41467-020-16257-x

Dobler, S. & Müller, J.K. (2000) Resolving phylogeny at the family level by mitochondrial cytochrome oxidase sequences: Phylogeny of carrion beetles (Coleoptera, Silphidae). *Molecular Phylogenetics and Evolution,* 15, 390-402. https://doi.org/10.1006/mpev.1999.0765

Dorsey, C.K. (1940) A comparative study of the larvae of six species of *Silpha*. *Annals of the Entomological Society of America,* 33, 120-139. https://doi.org/10.1093/aesa/33.1.120

Eggert, A.-K. & Müller, J.K. (1997) Biparental care and social evolution in burying beetles: Lessons from the larder. *The evolution of social behavior in insects and arachnids* (eds J.C. Choe & B.J. Crespi), pp. 216-236. Cambridge, Massachusetts, U.S.A, https://doi.org/10.1017/CBO9780511721953.011

Eggert, A.-K. & Müller, J.K. (2000) Timing of oviposition and reproductive skew in cobreeding female burying beetles (*Nicrophorus vespilloides*). *Behavioral Ecology,* 11, 357-366. https://doi.org/10.1093/beheco/11.4.357

Eggert, A.-K. & Müller, J.K. (2011) Timing of oviposition enables dominant female burying beetles to destroy brood-parasitic young. *Animal Behaviour,* 82, 1227-1233. https://doi.org/10.1016/j.anbehav.2011.09.001

Eggert, A.-K., Otte, T. & Müller, J.K. (2008) Starving the competition: A proximate cause of reproductive skew in burying beetles (*Nicrophorus vespilloides*). *Proceedings of the Royal Society B: Biological Sciences,* 275, 2521-2528. https://doi.org/10.1098/rspb.2008.0661

Eggert, A.-K., Reinking, M. & Müller, J.K. (1998) Parental care improves offspring survival and growth in burying beetles. *Animal Behaviour,* 55, 97-107. https://doi.org/10.1006/anbe.1997.0588

Evans, M.J., Wallman, J.F. & Barton, P.S. (2020) Traits reveal ecological strategies driving carrion insect community assembly. *Ecological Entomology,* 45, 966-977. https://doi.org/10.1111/een.12869

Felsenstein, J. (1985) Phylogenies and the comparative method. *The American Naturalist,* 125, 1-15. https://doi.org/10.1086/284325

García-Barros, E. (2000) Body size, egg size, and their interspecific relationships with ecological and life history traits in butterflies (Lepidoptera: Papilionoidea, Hesperioidea). *Biological Journal of the Linnean Society,* 70, 251-284. https://doi.org/10.1111/j.1095-8312.2000.tb00210.x

Gardner, A. & Smiseth, P.T. (2011) Evolution of parental care driven by mutual reinforcement of parental food provisioning and sibling competition. *Proceedings of the Royal Society B: Biological Sciences,* 278, 196-203. https://doi.org/10.1098/rspb.2010.1171

Gilbert, James D.J. & Manica, A. (2010) Parental care trade-offs and life-history relationships in insects. *The American Naturalist,* 176, 212-226. https://doi.org/10.1086/653661

Grafen, A. (1989) The phylogenetic regression. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 326, 119-157. https://doi.org/10.1098/rstb.1989.0106

Hamilton, W.D. (1964b) The genetical evolution of social behaviour. II. *Journal of Theoretical Biology,* 7, 17-52. https://doi.org/10.1016/0022-5193(64)90039-6

Holm, S. (1979) A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics,* 6, 65-70.

Hwang, W. & Lin, H.-M. (2013) Carcass fungistasis of the burying beetle *Nicrophorus nepalensis* Hope (Coleoptera: Silphidae). *Psyche: A Journal of Entomology,* 2013, 1-7. https://doi.org/10.1155/2013/162964

Ikeda, H., Kagaya, T., Kubota, K. & Abe, T. (2008) Evolutionary relationships among food habit, loss of flight, and reproductive traits: Life-history evolution in the Silphinae (Coleoptera: Silphidae). *Evolution,* 62, 2065-2079. https://doi.org/10.1111/j.1558-5646.2008.00432.x

Jarrett, B.J., Schrader, M., Rebar, D., Houslay, T.M. & Kilner, R.M. (2017) Cooperative interactions within the family enhance the capacity for evolutionary change in body size. *Nature Ecology & Evolution,* 1, 0178. https://doi.org/10.1038/s41559-017-0178

King, J.E., Riegler, M., Thomas, R.G. & Spooner-Hart, R.N. (2015) Phylogenetic placement of Australian carrion beetles (Coleoptera: Silphidae). *Austral Entomology,* 54, 366-375. https://doi.org/10.1111/aen.12138

Klug, H., Alonzo, S.H. & Bonsall, M.B. (2012) Theoretical foundations of parental care. *The evolution of parental care* (eds N.J. Royle, P.T. Smiseth & M. Kölliker), pp. 21-36. Oxford University Press, Oxford, U.K., https://doi.org/10.1093/acprof:oso/9780199692576.003.0002

Klug, H. & Bonsall, M.B. (2009) Life history and the evolution of parental care. *Evolution,* 64, 823-835. https://doi.org/10.1111/j.1558-5646.2009.00854.x

Klug, H. & Bonsall, M.B. (2014) What are the benefits of parental care? The importance of parental effects on developmental rate. *Ecology and Evolution,* 4, 2330-2351. https://doi.org/10.1002/ece3.1083

Kooijman, S.A.L.M., Lika, K., Augustine, S., Marn, N. & Kooi, B.W. (2020) The energetic basis of population growth in animal kingdom. *Ecological Modelling,* 428, 109055. https://doi.org/10.1016/j.ecolmodel.2020.109055

Kozol, A.J., Scott, M.P. & Traniello, J.F.A. (1988) The American burying beelte, *Nicrophorus americanus*: Studies on the natural history of declining species. *Psyche: A Journal of Entomology,* 95, 167-176. https://doi.org/10.1155/1988/79403

Kramer, J. & Meunier, J. (2019) The other facets of family life and their role in the evolution of animal sociality. *Biological Reviews,* 94, 199-215. https://doi.org/10.1111/brv.12443

Maddrell, S.H.P. (2018) How the simple shape and soft body of the larvae might explain the success of endopterygote insects. *Journal of Experimental Biology,* 221. https://doi.org/10.1242/jeb.177535

Maino, J.L., Kong, J.D., Hoffmann, A.A., Barton, M.G. & Kearney, M.R. (2016) Mechanistic models for predicting insect responses to climate change. *Current Opinion in Insect Science,* 17, 81-86. https://doi.org/10.1016/j.cois.2016.07.006

Marshall, D.J. & White, C.R. (2019) Have we outgrown the existing models of growth? *Trends in Ecology & Evolution,* 34, 102-111. https://doi.org/10.1016/j.tree.2018.10.005

Monteith, K.M., Andrews, C. & Smiseth, P.T. (2012) Post-hatching parental care masks the effects of egg size on offspring fitness: A removal experiment on burying beetles. *Journal of Evolutionary Biology,* 25, 1815-1822. https://doi.org/10.1111/j.1420-9101.2012.02567.x

Moses, Melanie E., Hou, C., Woodruff, William H., West, Geoffrey B., Nekola, Jeffery C., Zuo, W. & Brown, James H. (2008) Revisiting a model of ontogenetic growth: Estimating model parameters from theory and data. *The American Naturalist,* 171, 632-645. https://doi.org/10.1086/587073

Müller, J.K., Braunisch, V., Hwang, W. & Eggert, A.-K. (2007) Alternative tactics and individual reproductive success in natural associations of the burying beetle, *Nicrophorus vespilloides. Behavioral Ecology,* 18, 196-203. https://doi.org/10.1093/beheco/arl073

Müller, J.K. & Eggert, A.-K. (1990) Time-dependent shifts between infanticidal and parental behavior in female burying beetles: A mechanism of indirect mother-offspring recognition. *Behavioral Ecology and Sociobiology,* 27, 11-16. https://doi.org/10.1007/BF00183307

Müller, J.K., Eggert, A.-K. & Dressel, J. (1990) Intraspecific brood parasitism in the burying beetle, *Necrophorus vespilloides* (Coleoptera: Silphidae). *Animal Behaviour,* 40, 491-499. https://doi.org/10.1016/S0003-3472(05)80529-9

Otronen, M. (1988) The effect of body size on the outcome of fights in burying beetles. *Annales Zoologici Fennici,* 25, 191-201.

Peck, S.B. (1982) The life history of the Japanese carrion beetle *Ptomascopus morio* and the origins of parental care in *Nicrophorus* (Coleoptera, Silphidae, Nicrophorini). *Psyche: A Journal of Entomology,* 89, 107-111. https://doi.org/10.1155/1982/83023

Peters, R.H. & Wassenberg, K. (1983) The effect of body size on animal abundance. *Oecologia,* 60, 89-96. https://doi.org/10.1007/BF00379325

Prang, M.A., Zywucki, L., Körner, M. & Steiger, S. (2022) Differences in sibling cooperation in presence and absence of parental care in a genus with interspecific variation in offspring dependence. *Evolution,* 76, 320-331. https://doi.org/10.1111/evo.14414

Pukowski, E. (1933) Ȯkologische Untersuchungen an *Necrophorus* F. *Zeitschrift für Morphologie und Ökologie der Tiere,* 27, 518-586. https://doi.org/10.1007/BF00403155

Pütter, A. (1920) Studien über physiologische Ähnlichkeit VI. Wachstumsähnlichkeiten. *Pflüger's Archiv für die Gesamte Physiologie des Menschen und der Tiere,* 180, 298-340. https://doi.org/10.1007/BF01755094

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

Ratcliffe, B.C. (1972) The natural history of *Necrodes surinamensis* (Fabr.) (Coleoptera: Silphidae). *Transactions of the American Entomological Society,* 98.

Ratcliffe, B.C. (1996) The carrion beetles (Coleoptera: Silphidae) of Nebraska. *Bulletin of the University of Nebraska State Museum,* 13, 1-107. https://doi.org/10.1093/aesa/90.3.399

Rauter, C.M. & Moore, A.J. (2002) Quanititative genetics of growth and development time in the burying beetle *Nicrophorus pustulatus* in the presence and absence of post-hatching parental care. *Evolution,* 56, 96-110. https://doi.org/10.1111/j.0014-3820.2002.tb00852.x

Remeš, V., Matysioková, B. & Vrána, J. (2020) Adaptation and constraint shape the evolution of growth patterns in passerine birds across the globe. *Frontiers in Zoology,* 17, 1-13. https://doi.org/10.1186/s12983-020-00377-7

Ricklefs, R.E. (1984) The optimization of growth rate in altricial birds. *Ecology,* 65, 1602-1616. https://doi.org/10.2307/1939139

Ricklefs, R.E. (2003) Is rate of ontogenetic growth constrained by resource supply or tissue growth potential? A comment on West *et al.*'s model. *Functional Ecology,* 17, 384-393. https://doi.org/10.1046/j.1365-2435.2003.00745.x

Robinson, W.R., Peters, R.H. & Zimmermann, J. (1983) The effects of body size and temperature on metabolic rate of organisms. *Canadian Journal of Zoology,* 61, 281-288. https://doi.org/10.1139/z83-037

Roff, D.A. (2000) Trade-offs between growth and reproduction: An analysis of the quantitative genetic evidence. *Journal of Evolutionary Biology,* 13, 434-445. https://doi.org/10.1046/j.1420-9101.2000.00186.x

Royle, N.J., Smiseth, P.T. & Kölliker, M. (2012) *The evolution of parental care*. Oxford University Press, Oxford, U.K., https://doi.org/10.1093/acprof:oso/9780199692576.001.0001

Růžička, J., Jakubec, P., Mahlerová, K., Šípková, H. & Nishikawa, M. (2023) Integrative taxonomy and species distribution models of the genus *Diamesus* Hope, 1840 (Coleoptera: Staphylinidae: Silphinae). *Scientific Reports,* 13, 3192. https://doi.org/10.1038/s41598-023-30019-x

Satou, A., Nisimura, T. & Numata, H. (2001) Cost and necessity of parental care in the burying beetle *Nicrophorus quadripunctatus*. *Zoological Science,* 18, 975-979. https://doi.org/10.2108/zsj.18.975

Scott, M.P. (1990) Brood guarding and the evolution of male parental care in burying beetles. *Behavioral Ecology and Sociobiology,* 26, 31-39. https://doi.org/10.1007/BF00174022

Scott, M.P. (1998) The ecology and behavior of burying beetles. *Annual Review of Entomology,* 43, 595-618. https://doi.org/10.1146/annurev.ento.43.1.595

Scott, M.P. & Traniello, J.F.A. (1987) Behavioural cues trigger ovarian development in the burying beetle, *Nicrophorus tomentosus*. *Journal of Insect Physiology,* 33, 693-696. https://doi.org/10.1016/0022-1910(87)90053-9

Sibly, R.M. & Brown, J.H. (2020) Toward a physiological explanation of juvenile growth curves. *Journal of Zoology,* 311, 286-290. https://doi.org/10.1111/jzo.12770

Sikes, D.S., Madge, R.B. & Newton, A.F. (2002) A catalog of the Nicrophorinae (Coleoptera: Silphidae) of the world. *Zootaxa,* 65, 1-304. https://doi.org/10.11646/zootaxa.65.1.1

Sikes, D.S. & Venables, C. (2013) Molecular phylogeny of the burying beetles (Coleoptera: Silphidae: Nicrophorinae). *Molecular Phylogenetics and Evolution,* 69, 552-565. https://doi.org/10.1016/j.ympev.2013.07.022

Smiseth, P.T., Darwell, C.T. & Moore, A.J. (2003) Partial begging: An empirical model for the early evolution of offspring signalling. *Proceedings of the Royal Society B: Biological Sciences,* 270, 1773-1777. https://doi.org/10.1098/rspb.2003.2444

Smiseth, P.T., Kölliker, M. & Royle, N.J. (2012) What is parental care? *The evolution of parental care* (eds N.J. Royle, P.T. Smiseth & M. Kölliker), pp. 01-17. Oxford University Press, Oxford, U.K., https://doi.org/10.1093/acprof:oso/9780199692576.003.0001

Smiseth, P.T., Lennox, L. & Moore, A.J. (2007) Interaction between parental care and sibling competition: Parents enhance offspring growth and exacerbate sibling competition. *Evolution,* 61, 2331-2339. https://doi.org/10.1111/j.1558-5646.2007.00192.x

Starck, J.M. & Ricklefs, R.E. (1998) Patterns of development: The altricial-precocial spectrum. *Avian growth and development: Evolution within the altricial-precocial spectrum*, pp. 3-30. Oxford University Press, Oxford, U.K., ISBN: 9780195106084

Stearns, S.C. (1992) *The evolution of life histories*. Oxford University Press, Oxford, U.K., ISBN: 9780198577416

Steiger, S. (2013) Bigger mothers are better mothers: Disentangling size-related prenatal and postnatal maternal effects. *Proceedings of the Royal Society B: Biological Sciences,* 280, 20131225. https://doi.org/10.1098/rspb.2013.1225

Summers, K., McKeon, C.S. & Heying, H. (2006) The evolution of parental care and egg size: A comparative analysis in frogs. *Proceedings of the Royal Society B: Biological Sciences,* 273, 687-692. https://doi.org/10.1098/rspb.2005.3368

Suzuki, S. (1999) Does carrion-burial by *Nicrophorus vespilloides* (Silphidae: Coleoptera) prevent discovery by other burying beetles? *Entomological Science,* 2, 205-208. ISSN: 13438786

Suzuki, S. (2000) Carrion burial by *Nicrophorus vespilloides* (Coleoptera: Silphidae) prevents fly infestation. *Entomological Science,* 3, 269-272.

Suzuki, S. (2001) Suppression of fungal development on carcasses by the burying beetle *Nicrophorus quadripunctatus* (Coleoptera: Silphidae). *Entomological Science,* 4, 403-405.

Suzuki, S. & Nagano, M. (2006) Resource guarding by *Ptomascopus morio*, simple parental care in the Nicrophorinae. *European Journal of Entomology,* 103, 245-248. https://doi.org/10.14411/eje.2006.027

Talloen, W., Van Dyck, H. & Lens, L. (2004) The cost of melanization: Butterfly wing coloration under environmental stress. *Evolution,* 58, 360-366. https://doi.org/10.1111/j.0014-3820.2004.tb01651.x

Trumbo, S.T. (1990) Interference competition among burying beetles (Silphidae, *Nicrophorus*). *Ecological Entomology,* 15, 347-355. https://doi.org/10.1111/j.1365-2311.1990.tb00816.x

Trumbo, S.T. (1992) Monogamy to communal breeding: Exploitation of a broad resource base by burying beetles (*Nicrophorus*). *Ecological Entomology,* 17, 289-298. https://doi.org/10.1111/j.1365-2311.1992.tb01060.x

Trumbo, S.T. (2016) Carcass age and reproductive costs for *Nicrophorus orbicollis* (Coleoptera: Silphidae). *Environmental Entomology,* 45, 1178-1183. https://doi.org/10.1093/ee/nvw114

Trumbo, S.T., Kon, M. & Sikes, D. (2001) The reproductive biology of *Ptomascopus morio*, a brood parasite of *Nicrophorus*. *Journal of Zoology,* 255, 543-560. https://doi.org/10.1017/S0952836901001637

Trumbo, S.T., Philbrick, P.K.B., Stökl, J. & Steiger, S. (2021) Burying beetle parents adaptively manipulate information broadcast from a microbial community. *The American Naturalist,* 197, 366-378. https://doi.org/10.1086/712602

Trumbo, S.T. & Robinson, G.E. (2004) Nutrition, hormones and life history in burying beetles. *Journal of Insect Physiology,* 50, 383-391. https://doi.org/10.1016/j.jinsphys.2004.01.008

Trumbo, S.T. & Robinson, G.E. (2008) Social and nonsocial stimuli and juvenile hormone titer in a male burying beetle, *Nicrophorus orbicollis*. *Journal of Insect Physiology,* 54, 630-635. https://doi.org/10.1016/j.jinsphys.2007.12.012

Trumbo, S.T. & Sikes, D.S. (2021) Resource concealment and the evolution of parental care in burying beetles. *Journal of Zoology,* 315, 175-182. https://doi.org/10.1111/jzo.12916

Trumbo, S.T. & Steiger, S. (2020) Finding a fresh carcass: Bacterially derived volatiles and burying beetle search success. *Chemoecology,* 30, 287-296. https://doi.org/10.1007/s00049-020-00318-0

Trumbo, S.T. & Valletta, R.C. (2007) The costs of confronting infanticidal intruders in a burying beetle. *Ethology,* 113, 386-393. https://doi.org/10.1111/j.1439-0310.2006.01326.x

Trumbo, S.T. & Xhihani, E. (2015) Mass–size relationships, starvation and recovery in an engorging feeder. *Physiological Entomology,* 40, 257-263. https://doi.org/10.1111/phen.12110

von Bertalanffy, L. (1938) A quantitative theory of organic growth (inqiuries on growth laws II). *Human Biology,* 10, 181-213. https://doi.org/10.2307/41447359

von Lengerken, H. (1938) Studien über die Lebenserscheinungen der Silphini (Coleopt.). *Zeitschrift für Morphologie und Ökologie der Tiere,* 33, 654-666. https://doi.org/10.1007/BF00407572

Wesołowski, T. (1994) On the origin of parental care and the early evolution of male and female parental roles in birds. *The American Naturalist,* 143, 39-58. https://doi.org/10.1086/285595

West, G.B., Brown, J.H. & Enquist, B.J. (2001) A general model for ontogenetic growth. *Nature,* 413, 628-631. https://doi.org/10.1038/35098076

Williams, T.D. (1994) Intraspecific variation in egg size and egg composition in birds: Effects on offspring fitness. *Biological Reviews of the Cambridge Philosophical Society,* 69, 35-59. https://doi.org/10.1111/j.1469-185x.1994.tb01485.x

Wilson, D.S. (1975) The adequacy of body size as a niche difference. *The American Naturalist,* 109, 769-784. https://doi.org/10.1086/283042

Publication 2: Offspring dependence on parental care and the role of parental transfer of oral fluids in burying beetles

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S.S. conceived the study. A.C.-N. and S.S. designed the study. A.C.-N. and M.A.P. performed the experiments. A.C.-N. and M.A.P. analysed the data. A.C.-N., M.A.P., S.T.T., H.V., A.-K.E., S.K.S., and S.S. discussed the results. A.C.N. wrote a draft manuscript and M.A.P., S.T.T., H.V., A.-K.E., S.K.S., and S.S. contributed substantially to revision. All authors read and approved the final manuscript.

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Abstract

Background

Immature stages of many animals can forage and feed on their own, whereas others depend on their parents' assistance to obtain or process food. But how does such dependency evolve, and which offspring and parental traits are involved? Burying beetles (*Nicrophorus*) provide extensive biparental care, including food provisioning to their offspring. Interestingly, there is substantial variation in the reliance of offspring on post-hatching care among species. Here, we examine the proximate mechanisms underlying offspring dependence, focusing on the larvae of *N. orbicollis*, which are not able to survive in the absence of parents. We specifically asked whether the high offspring dependence is caused by (1) a low starvation tolerance, (2) a low ability to self-feed or (3) the need to obtain parental oral fluids. Finally, we determined how much care (i.e. duration of care) they require to be able to survive.

Results

We demonstrate that *N. orbicollis* larvae are not characterized by a lower starvation tolerance than larvae of the more independent species. Hatchlings of *N. orbicollis* are generally able to self-feed, but the efficiency depends on the kind of food presented and differs from the more independent species. Further, we show that even when providing highly dependent *N. orbicollis* larvae with easy ingestible liquefied mice carrion, only few of them survived to pupation. However, adding parental oral fluids significantly increased their survival rate. Finally, we demonstrate that survival and growth of dependent *N. orbicollis* larvae is increased greatly by only a few hours of parental care.

Conclusions

Considering the fact that larvae of other burying beetle species are able to survive in the absence of care, the high dependence of *N. orbicollis* larvae is puzzling. Even though they have not lost the ability to self-feed, an easily digestible, liquefied carrion meal is not sufficient to ensure their survival. However, our results indicate that the transfer of parental oral fluids is an essential component of care. In the majority of mammals, offspring rely on the exchange of fluids (i.e. milk) to survive, and our findings suggest that even in subsocial insects, such as burying beetles, parental fluids can significantly affect offspring survival.

Keywords

Nicrophorus, Burying beetles, Parental care, Trophallaxis, Starvation tolerance, Oral secretions, Offspring dependence

Background

Most animals eat to acquire nutrients that are essential to fulfil the energetic needs for their growth or reproduction (Slansky 1982; Behmer 2009). Generally, only a part of the food an individual consumes is absorbed, metabolised and converted into usable energy or nutrients, i.e. 'digestive efficiency' (Scriber & Slansky 1981; Castro, Stoyan & Myers 1989; Karasov 1990; Bairlein 1999). Digestive efficiency reflects how effectively an individual can exploit food resources, and this might vary between species due to different capabilities to process and absorb food when the resource is very challenging, or due to different physiological requirements, irrespective of the type of food (Bairlein 1999). To overcome these challenges, parents in various animal taxa have evolved traits to provision offspring with food or to assist them with digestion. In mammals, for example, parental care is obligate. Females provide milk (Clutton-Brock 1991; Balshine 2012), which is not only rich in lipids and proteins, but also contains bioactive components such as growth factors, hormones, or immunological factors that contribute to the development and protection of the young (Ballard & Morrow 2013). In pigeons, parents produce and feed their chicks 'crop milk' that contains nutrients, minerals and growth factors (Shetty *et al.* 1992), as well as immune-active compounds such as immunoglobulins (Engberg *et al.* 1992) and carotenoids (Eraud *et al.* 2008). Thus, food provisioning not only entails the breakdown and pre-digestion of food, but also the transfer of important ancillary compounds.

Unlike mammals or birds, parental food provisioning occurs in only about 1 % of insect species (Royle, Russell & Wilson 2014). Researchers have repeatedly suggested that when food is ephemeral (e.g., carrion, dung) or difficult to process (e.g., wood), insects are more likely to evolve some form of parental care, such as facilitating feeding of offspring, or protecting both the resource and the offspring from competitors, predators, or parasites (Tallamy & Wood 1986; Wong, Meunier & Kölliker 2013). Wood roaches of the genus *Salganea*, for example, have evolved morphological adaptations of the mouthparts to facilitate the up-take of stomodeal substances via trophallaxis by the mother (Costa 2006; Maekawa, Matsumoto & Nalepa 2008; Nalepa *et al.* 2008). However, it is currently unclear whether these substances contain wood fragments, nutrients, and/or enzymes or other chemicals involved in the degradation of cellulose (Shimada &

Maekawa 2011). Generally, oral fluids exchanged by trophallaxis may include proteins that are regulators of growth, development, and behavioural maturation (LeBoeuf *et al.* 2016; LeBoeuf 2017). Alternatively, parents might pass symbionts to offspring that are essential for their survival and growth. Altricial neonates of the wood-feeding cockroach *Cryptocercus punctulatus*, for example, cannot directly process wood, but instead rely on the hindgut fluids of their parents to acquire symbionts that are necessary for digestion (i.e. proctodeal trophallaxis) (Nalepa & Bell 1997).

In contrast to wood or foliage, carrion is a highly nutritious and ephemeral resource that is easily digested (Tallamy & Wood 1986). Dipteran females are usually the first insects to arrive at a carcass and to deposit their eggs or first-instar larvae directly on top of the carrion, often in natural body openings or at wound sites (Byrd 2002). The maggots then, without any parental assistance, immediately start feeding on the carrion at the site where they emerge. Most beetles in the family Silphidae also have larvae that depend on carrion as their food, and in all genera except *Nicrophorus*, the larvae feed independently. Only in *Nicrophorus* do adult beetles bury carcasses in an apparent attempt to monopolize and defend them for their young. In addition, parents provide food to their offspring and, within this genus, there is significant variation in the dependence of offspring on post-hatching parental care, most likely on parental feeding (Trumbo 1992; Capodeanu-Nägler *et al.* 2016). The larvae of some species can easily feed and survive on a carcass without parental help, whereas others cannot. However, the evolutionary causes driving these differences in the dependence on parental care among species that utilize the same food resource remain obscure. One mathematical model for the evolution of parental care predicts that in species that provide care to their offspring, food provisioning is expected to evolve if it is more efficient than offspring self-feeding, or more efficient than parental efforts to guard against predators (Gardner & Smiseth 2011). Also, the evolution of food provisioning promotes a mutual reinforcement between parental feeding and sibling competition, resulting in a unidirectional trend from no to full parental food provisioning (Gardner & Smiseth 2011). Once parental feeding has evolved, coadaptation between parental and offspring traits may lead to the delayed ontogenetic development of traits that are necessary for offspring self-feeding (Capodeanu-Nägler *et al.* 2016).

However, to better understand which factors drive the evolutionary loss of independence, it is crucial to determine the proximate cause of offspring dependence. On what parental service do offspring actually rely, and which offspring traits differ between dependent and independent species? Are there insect hatchlings that have lost their ability to self-feed similar to neonates of altricial mammals and birds?

Here, we examine the proximate mechanisms underlying offspring dependence using burying beetles as a model system. Burying beetles are well-known for their habit of interring small vertebrate carcasses and providing extensive biparental care to their offspring before and after hatching (Pukowski 1933; Eggert & Müller 1997; Scott 1998). In *N. vespilloides*, larvae are capable of self-feeding, but nevertheless beg for regurgitated pre-digested carrion from their parents (Smiseth & Moore 2002; Smiseth, Darwell & Moore 2003). Parental regurgitations are hypothesized to ensure a sufficient food supply for larvae, when their mandibles are still soft and not fully sclerotized following larval moults (Pukowski 1933). In our previous study, we found that offspring of the three species *N. orbicollis, N. pustulatus*, and *N. vespilloides* show marked differences in their dependence on parental provisioning, or at least on post-hatching care (Capodeanu-Nägler *et al.* 2016). *N. orbicollis*, which is one of the most basal species within the genus *Nicrophorus* (Sikes & Venables 2013), appears to be a beetle with obligatory parental care, as offspring do not survive in the absence of parents, whereas parental care is facultative in *N. pustulatus* and *N. vespilloides* (Trumbo 1992; Capodeanu-Nägler *et al.* 2016). Likewise, parental care appears to be facultative in many other *Nicrophorus* species, including *N. mexicanus* (Anduaga & Huerta 2001), *N. defodiens, N. tomentosus* (Trumbo 1992) and *N. quadripunctatus* (Satou, Nisimura & Numata 2001). This raises the question, therefore, as to why *N. orbicollis* is so exceptional among other *Nicrophorus* species with regard to offspring dependency, and more particularly, what causes the striking helplessness of offspring in the absence of parents. As our study aimed to investigate the proximate mechanisms of offspring dependency, we focused on the most dependent species studied to date, *N. orbicollis*, and drew comparisons to more independent species when required.

We first tested the hypothesis that larvae of the different species are equally efficient at self-feeding, but that *N. orbicollis* parents may invest fewer resources into eggs or their larvae may be fast metabolisers. Both scenarios would result in larvae with a higher food demand in a shorter time period. To test this hypothesis, we did not measure nutrient content in eggs nor the metabolic rate of larvae, but rather used starvation tolerance of larvae as a proxy for a high resource need per time unit. In addition, we noted larval mass at hatching as an indicator of egg investment. In the next step, we investigated whether *N. orbicollis* larvae are capable of self-feeding from the time of hatching, or whether the expression of morphological adaptations needed for self-feeding is delayed compared to more independent species. Additionally, we tested whether the self-feeding capacity of larval *N. orbicollis* is inferior to the self-feeding capacity of the more independent species. In burying beetles, parental food provisioning entails not only the regurgitation of partially digested carrion, but also the transfer of oral fluids, which might also contain important microbial symbionts, enzymes, or growth hormones (see e.g. LeBoeuf 2017). Thus, upon discovering that larval *N. orbicollis* are particularly effective in feeding on pieces of baby mice, we tried to rear them on an easily digestible diet of liquefied carrion either supplemented with parental oral secretions or not. With this experiment, we also tested the hypothesis that the characteristics of larval mandibles play an important role in determining offspring dependence. In *N. vespilloides*, a previous study has shown that larvae that receive at least 12 h of parental care survive well, and average larval mass does not significantly increase with longer care (Eggert, Reinking & Müller 1998). In a final experiment, we therefore attempted to determine the minimum duration of post-hatching care required for larval survival to adulthood in *N. orbicollis*.

Methods

Origin and maintenance of experimental beetles

N. vespilloides used in the study were descendants of beetles collected from carrion-baited pitfall traps in a forest near Ulm, Germany (48°25′03″N, 9°57′45″E). Cultures of *N. pustulatus* and *N. orbicollis* were established at Ulm University from outbred colonies maintained at the Institute of Zoology, University of Freiburg, Germany. We maintained outbred colonies of both species by introducing beetles captured in baited pitfall traps established in a forested area near Lexington, Illinois, U.S.A. (40°39′57″N, 88°53′49″W). All beetles were held in temperature-controlled incubators at 20 °C on a 16:8 h light:dark cycle. Before the experiments, groups of up to five adult beetles of the same sex and family of each species were kept in small plastic containers (10 × 10 cm and 6 cm high) filled with moist peat. Beetles were fed freshly decapitated mealworms *ad libitum* twice a week. At the time of experiments, beetles were virgin and between 20 and 30 days of age.

Experimental design

Experiment 1: Starvation tolerance of larvae

Larval *N. orbicollis* do not survive in the absence of post-hatching care (Trumbo 1992; Capodeanu-Nägler *et al.* 2016). In this experiment, we measured starvation tolerance of *N. orbicollis* offspring in comparison with the more independent species, *N. pustulatus* and *N. vespilloides*. For this, we randomly selected non-sibling pairs of male and female beetles, placed them in small plastic containers filled with peat (10 × 10 cm and 6 cm high), and induced reproduction by providing them with a 20 g (± 3 g) thawed mouse carcass (Frostfutter.de – B.A.F Group GmbH, Germany).

In the case of the nocturnal species, *N. orbicollis* and *N. pustulatus*, mice were provided during the dark portion of the photoperiod, whereas for crepuscular *N. vespilloides*, mice were provided during the light portion. To prevent hatching larvae from access to food, we transferred parents and the carcass to new boxes filled with peat after the egg-laying period (see Capodeanu-Nägler *et al.* 2016), and left the eggs to hatch in the old container. From the expected time of larval hatching, we checked for larvae every hour. Then, for each species, we set up a minimum number of 30 larvae from at least six different families (number of larvae, families: *N. orbicollis*: 36, 6; *N. pustulatus*: 33, 11; *N. vespilloides*: 39, 9) to avoid any family effect on larval survival. One larva each was added on top of a moistened paper tissue in a shallow plastic tray (3 × 3 cm × 0.5 cm high) without access to food, and kept in a temperature-controlled room at 20 °C. We then checked for the survival of each larva every hour up to a maximum of 42 h, and moistened the paper tissue, if necessary, to ensure an adequate supply of water to the larvae. Finally, we recorded the number of hours that larvae survived.

Experiment 2: Self-feeding ability on different food resources

Here, we set up non-sibling pairs of beetles as in experiment 1 for the three species, *N. orbicollis*, *N. pustulatus*, and *N. vespilloides*. As before, parents and their carcass were transferred to new boxes after the egg laying period, and the old boxes were checked at least every 8 h for the hatching of larvae. To measure interspecific variation in the ability of larvae to self-feed when parents are absent, we established three treatments per species (n = 15 for each species and treatment) in which we offered individual larvae one of three different food resources ranging from very challenging food to very easily accessible and digestible food: (1) carrion prepared by parents in the pre-hatching period without a hole created by parents; (2) carrion prepared by parents in the pre-hatching period with a hole in the carcass created by parents; (3) small pieces of baby mice. Generally, burying beetle parents create an opening in the carcass shortly before or after larval hatching, allowing larvae direct access to the food (Eggert, Reinking & Müller 1998). To ensure that we obtained approximately equal numbers of prepared carcasses with (n = 15) and without a hole (n = 15), we set up additional pairs for reproduction in each species. Thus, for treatments 1 and 2, we provided 50 pairs in each species with a 20 g (± 3 g) thawed mouse carcass and allowed them to provide pre-hatching care according to their species-specific duration (*N. orbicollis*: 120 h; *N. pustulatus*: 80 h; *N. vespilloides*: 70 h). We then inspected prepared carcasses for an opening in the integument and assigned them to the treatment ‘prepared carrion without hole’ in those instances where there was no hole. Carcasses that had already been processed and opened by the parents were additionally cut open using scissors and assigned to the treatment ‘prepared carrion with hole’. All food resources were offered in small plastic containers without peat (10 × 10 cm and 6 cm high), but lined with moist paper tissue. As soon as the larvae hatched, their initial mass (0 h) was determined to 0.01 mg using a precision scale (Kern ABT 220-5DM, Kern und Sohn GmbH, Balingen, Germany) before allowing them access to a food resource. Immediately thereafter, one larva each was randomly added on top of one of the three food resources. Larvae were then weighed again 2 h later to detect any changes in larval mass during this time interval.

Experiment 3: Effect of oral secretions on larval *N. orbicollis*

Here, we determined whether larvae of the most dependent species, *N. orbicollis*, could be reared in the absence of their parents when provided with a liquidized paste of baby mice mixed with or without oral fluids of their parents. For this, we established 40 pairs of male and female beetles, 20 of which were set up 2 days in advance and used for the extraction of oral secretions. The other 20 pairs served to provide larvae for the actual experiment. As before, parents and the carcass were transferred to new boxes after the egg-laying period, leaving the eggs in the old boxes to hatch. We established two treatment groups in which we provided larvae with (1) a paste of baby mice that included oral secretions of care-giving male or female parents that had been given access to larvae and a carcass for 24–48 h (n = 35), or (2) a paste of baby mice without oral secretions of parents (n = 35). To prepare the paste of baby mice, we placed 30 dead and frozen baby mice (1–3 g; Frostfutter.de—B.A.F Group GmbH, Germany) into a blender together with 30 mL of water, and mixed them until the paste was homogenous. To obtain the regurgitated oral fluids from a parent, we gently squeezed the thoracic-abdominal region of a beetle with a pair of forceps and collected the secretions with a pipette. For the experiments, we placed 5 larvae from one family (n = 7 for each treatment) that had hatched at the same time together in a petri dish containing a moist paper tissue. We then checked for the survival of larvae three times a day, and exchanged both the moist paper tissue and the food when larvae were still alive. We recorded the number of hours each larva survived.

In the group including oral secretions of parents (1), larvae were provided with 5 µL of oral secretions directly added on top of the moist paper tissue for the first 24 h. In addition, we added two 0.2 mL Eppendorf tubes containing 5 mg of baby mouse paste mixed with 5 µL of oral secretions of a parental beetle. Oral secretions were always freshly obtained from the parental beetles. In the group without oral secretions (2), larvae in the first 24 h were only provided with two 0.2 mL Eppendorf tubes that contained approximately 5 mg of baby mouse paste. The

Eppendorf tubes in both treatments were sliced open at both ends to facilitate ready access of larvae to the food. After 24 h, larvae of both treatments received one 0.5 mL Eppendorf tube containing baby mouse paste without oral secretions. After 48 h, larvae received one opened baby mouse carcass and one 0.5 mL Eppendorf tube containing baby mouse paste without oral secretions. As parental regurgitations in *N. orbicollis* substantially decrease after 48 h, and larval survival and mass are subsequently not reduced in the absence of care (Fetherston, Scott & Traniello 1990; Scott & Traniello 1990, see also experiment 4), we opted to provide larvae solely with mouse carcasses thereafter. After 120 h, surviving larvae were placed into boxes with soil and provided with two opened baby mouse carcasses in succession, the first of which was left for 8 h, after which it was exchanged with the second carcass for an additional 8 h. This was done to ensure a sufficient food supply for larvae just prior to pupation. After the second carcass was removed, the larvae were left to pupate.

Experiment 4: Duration of post-hatching care needed to ensure development of larval *N. orbicollis*

The aim of this experiment was to determine the minimum duration of post-hatching care needed to ensure survival of larvae. To test this, we set up 200 non-sibling pairs of *N. orbicollis* beetles as in the previous experiments. After the egg laying period (see Capodeanu-Nägler *et al.* 2016), parents and their carcass were transferred to new boxes, and the old boxes were checked every 8 h for the hatching of larvae. To control for variation between families and individual differences in behaviour (Rauter & Moore 1999), we provided each pair of beetles with a brood of 15 newly hatched larvae of mixed parentage (Arce *et al.* 2012; Capodeanu-Nägler *et al.* 2016), see also (Rauter & Moore 2004)). Burying beetles exhibit temporally-based kin discrimination in which they kill any larvae arriving on the carcass before their own eggs would have hatched (Müller & Eggert 1990). Hence, we only provided pairs with larvae after their own larvae had begun hatching. The larvae were placed directly onto the carcass, in which we had cut a hole through the skin earlier to facilitate larval access to the carrion in each of the treatments. We then allowed parents to provide post-hatching care for 1 h, 3 h, 6 h, 12 h, 24 h, or 48 h (n = 15 or 16 per treatment). In addition, we established a 'pre-hatching care' treatment (n = 15), in which parents were only allowed to prepare the carcass, but were pre- vented from providing post-hatching care ('0 h'). Finally, we also established a 'full-care' treatment, in which parents were allowed to prepare the carcass and to provide post-hatching care until larvae dispersed (8 ± 2 days). As soon as the surviving larvae of each brood left the carcass for pupation, they were counted and weighed.

Statistical analysis

All data were analysed and plotted using R version 3.1.2 (R Core Team 2014) or SPSS version 21.0 (Chicago, IL, USA). For experiments1 and 3, we used the Kaplan-Meier method in SPSS to estimate survival of larvae as a function of time. To test for differences in larval survival between the three species in experiment 1 and the two treatments in experiment 3, we used a log-rank test in SPSS. For experiment 2, we used the relative change in larval mass between 0 h and 2 h as a proxy to assess the ability to self-feed in each species. As larval mass at hatching differed among species (GLM with Gaussian errors: $F_{2,177} = 517.69$, $P < 0.001$), we first divided the absolute change in larval mass by the mass of each larva at hatching. We then applied generalised linear models (GLMs) with Gaussian distribution with species, treatment and species*treatment as fixed factors and the relative change in larval mass as the dependent variable. To identify species-specific treatment effects, we continued with GLMs followed by pairwise comparisons with Bonferroni correction for multiple testing within each of the three species in which treatment was included as a fixed factor and the relative change in larval mass as the dependent variable. For experiment 4, we included duration of post-hatching care (0 h, 1 h, 3 h, 6 h, 12 h, 24 h, 48 h, full care) as a fixed factor, and the absolute number of larvae that survived and mean larval mass per brood as dependent variables. We then applied GLMs with Poisson distribution followed by pairwise comparisons with Bonferroni correction for multiple testing. In addition, we compared the mean larval mass per brood at dispersal by using a GLM with Gaussian distribution.

Results

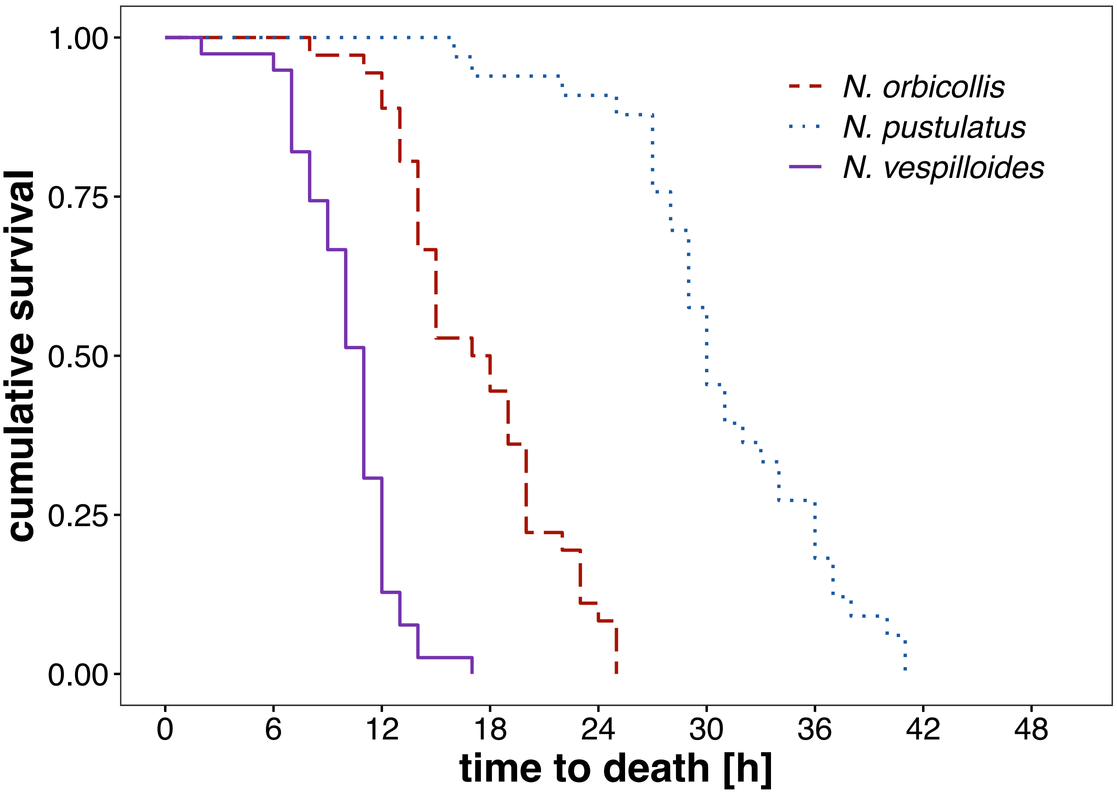

Figure 2.1
Survival of larvae without access to food in *N. orbicollis* (N = 36), *N. pustulatus* (N = 33), and *N. vespilloides* (N = 39) [h]. Kaplan-Meier estimated survival curves. (© Figure adapted from Capodeanu-Nägler *et al.* (2018))

Experiment 1: Starvation tolerance of larvae

Survival of larvae without access to food varied significantly among the three species (log-rank test, for all: $P < 0.001$, see **Fig. 2.1**). On average, highly dependent *N. orbicollis* larvae survived longer (mean: 17.44 ± SE 0.75 h) than larval *N. vespilloides* (mean: 10.21 ± SE 0.43 h) which show an intermediate dependence on parental care (log-rank test, $x^2 = 54.\ 28$, $P < 0.001$). However, *N. orbicollis* larvae survived significantly shorter than the highly independent *N. pustulatus* larvae (mean: 30.82 ± SE 1.04 h) (log-rank test, $x^2 = 62.53$, P < 0.001). *N. pustulatus* larvae also survived longer than larval *N. vespilloides* (log-rank test, $x^2 = 77.03$, $P < 0.001$).

Table 2.1

Results of the GLM of the effect of species (*N. orbicollis, N. pustulatus, N. vespilloides*), treatment (prepared carrion without hole, prepared carrion with hole, baby mice, overall sample size = 135) and the interaction of species and treatment on relative change in larval mass.

factor	relative change in larval mass		
	df	*F*	*P*
species	2	13.44	< **0.001**
treatment	2	17.61	< **0.001**
species * treatment	4	6.00	< **0.001**

Significant p-values are typed in bold.

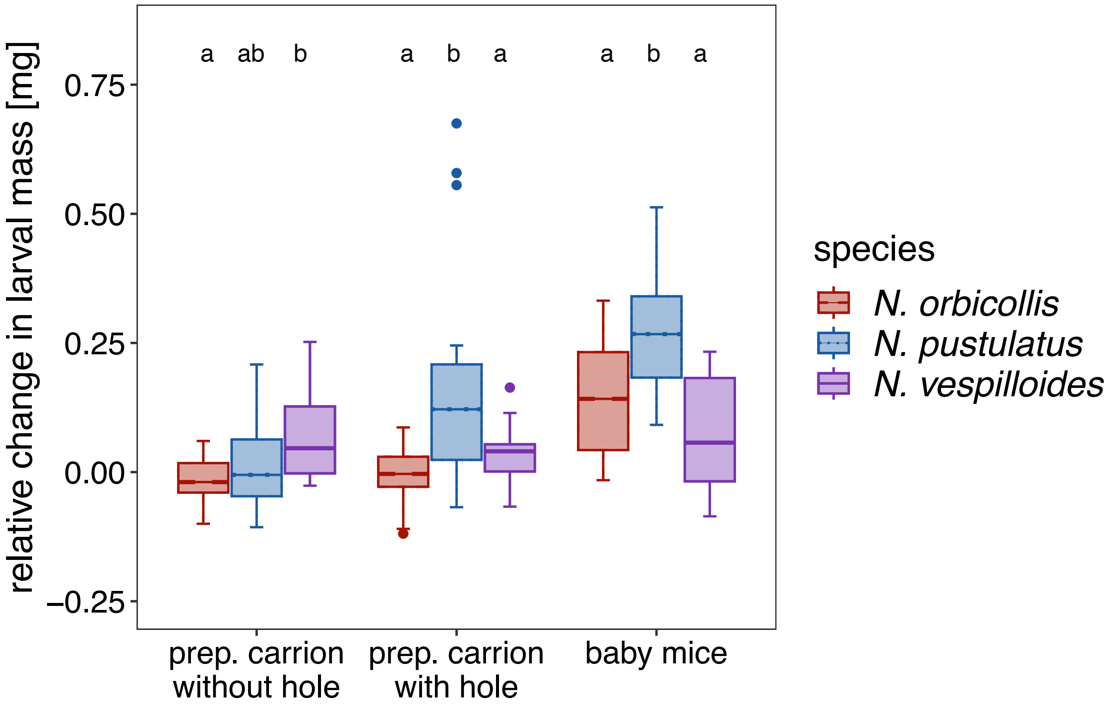

Figure 2.2

The change in larval mass from hatching to two hours after hatching in *N. orbicollis*, *N. pustulatus*, and *N. vespilloides* on different food sources [mg]. N = 15 for each species and treatment. Boxplots show median, interquartile range, minimum/maximum range. The dots are values that fall outside the interquartile range (> 1.5× interquartile range). Different letters indicate significant differences between species within one treatment. (© Figure adapted from Capodeanu-Nägler *et al.* (2018))

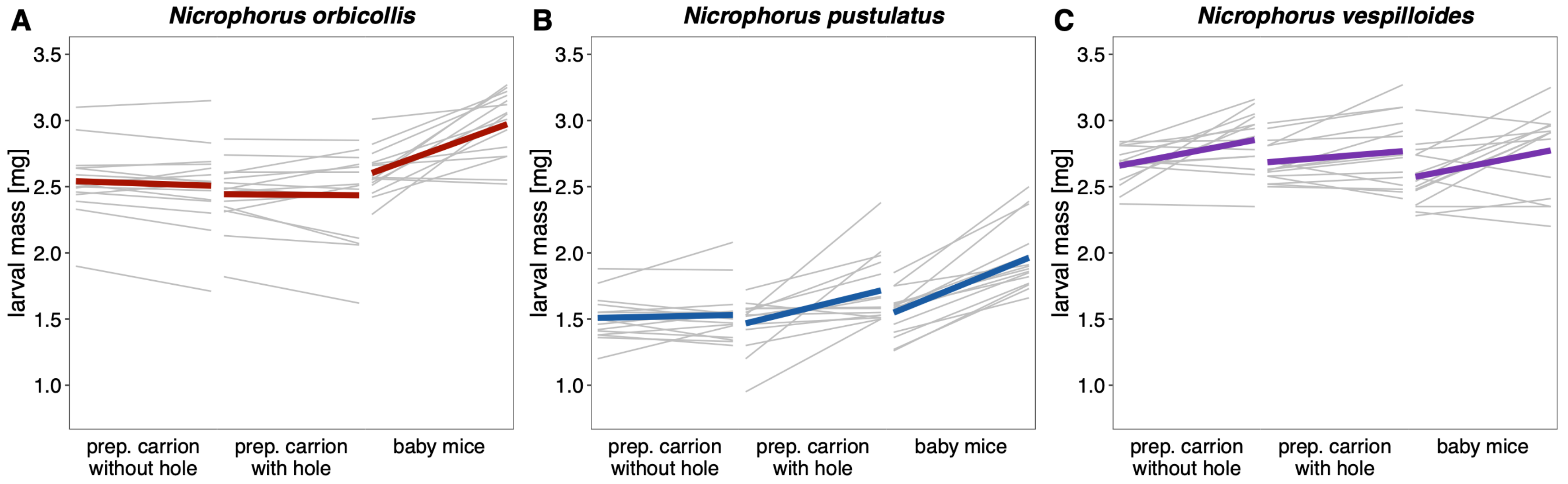

Figure 2.3
Larval masses [mg] at hatching (0 h) and after two hours on a specific food source in (**a**) *N. orbicollis*, (**b**) *N. pustulatus* and (**c**) *N. vespilloides*. N = 15 for each species and treatment. Each line represents one individual larva. The red line represents the mean of all larvae in one treatment. (© Figure adapted from Capodeanu-Nägler *et al.* (2018))

Experiment 2: Self-feeding ability on different food resources

When we provided individual larvae with one of three different food resources, we found significant effects of treatment and species as well as a significant interaction on the relative change in larval mass (**Table 2.1**, see **Fig. 2.2**). Across all treatments, highly dependent *N. orbicollis* larvae gained less larval mass (mean 0.04 ± SE 0.02 mg) than independent *N. pustulatus* (mean 0.16 ± SE 0.03 mg) (Pairwise test: $P < 0.001$). There was no difference in the change in larval mass between *N. orbicollis* and *N. vespilloides* (mean: 0.06 ± SE 0.01 mg) (Pairwise test: $P = 1.00$). The gain in larval mass was higher in *N. pustulatus* than in *N. vespilloides* (Pairwise test: $P = 0.003$). The type of food had an effect on change in larval mass, but this effect differed among the species (**Table 2.1**, see **Fig. 2.2**). *N. orbicollis* and *N. pustulatus* exhibited increased mass when provided with baby mice, whereas larval *N. vespilloides* did not. Also, cutting a hole into the carrion had a clear positive effect on weight increase in larval *N. pustulatus*, but not in the other two species.

To better understand how the type of food affected the relative change in larval mass, we analysed each species separately. We found that the change in larval mass depended on the food provided in *N. orbicollis* and *N. pustulatus* (GLM with Gaussian errors: $F_{2,42} = 20.52$, P < 0.001 for *N. orbicollis*; $F_{2,42} = 10.10$, $P < 0.001$ for *N. pustulatus*), but not in *N. vespilloides* (GLM with Gaussian errors: $F_{2,42} = 1.36$, $P = 0.27$). In *N. orbicollis*, larvae only gained weight when provided with baby mice (Pairwise test: $P < 0.001$ for baby mice vs. prepared carrion with hole, baby mice vs. prepared carrion without hole, **Fig. 2.3A**). In contrast to highly dependent *N. orbicollis*, larvae of the more independent species were able to gain weight when provided with a carcass that was prepared by the parents. In *N. pustulatus*, larvae showed a greater increase in mass when provided with a prepared carcass with a hole (Pairwise test: $P = 0.01$) or baby mice than when provided with a prepared carcass without a hole (Pairwise test: $P < 0.001$, **Fig. 2.3B**). Larval *N. vespilloides* gained weight equally on the different types of food (see **Fig. 2.3C**).

Further, we should note that larval mass at hatching differed significantly among the three species (GLM with Gaussian errors: $F_{2,177} = 517.69$, $P < 0.001$). On average, larval *N. vespilloides* were heavier than larval *N. orbicollis* and larval *N. pustulatus* at hatching (Pairwise test: for both, $P < 0.001$). Larval *N. orbicollis* were, in turn, heavier than larval *N. pustulatus* (Pairwise test: $P < 0.001$).

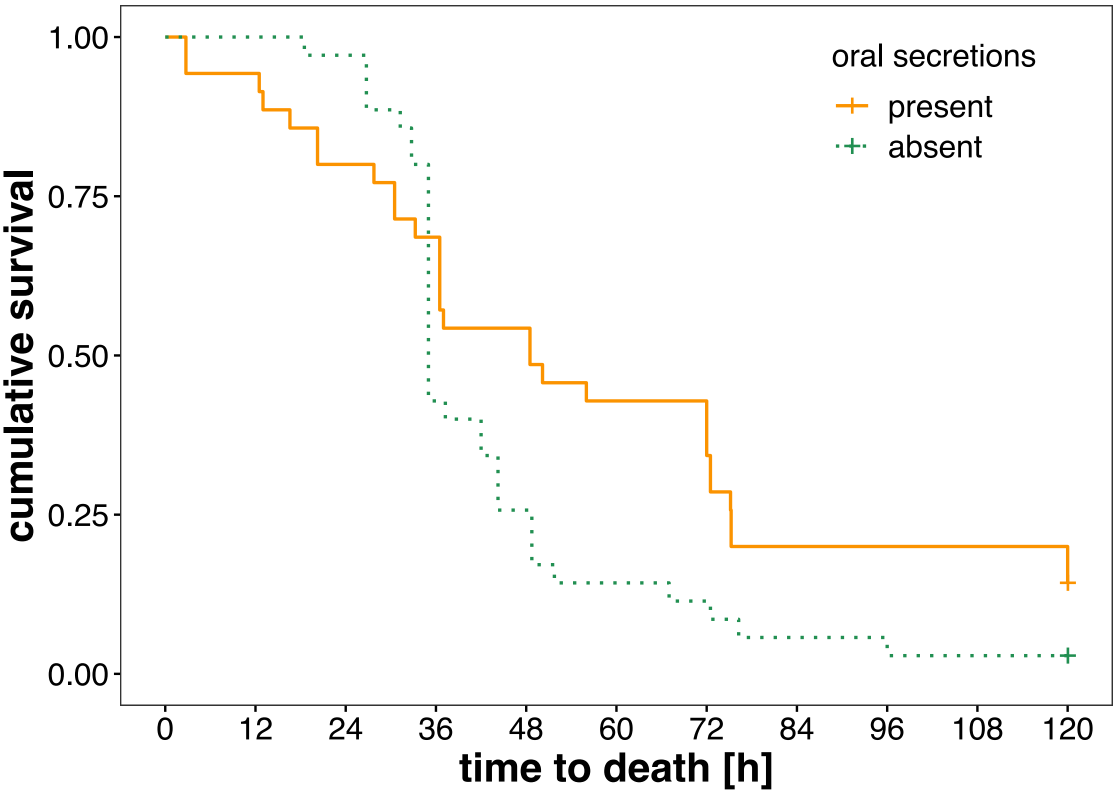

Figure 2.4
Survival of *N. orbicollis* larvae that received a paste of baby mice with or without oral secretions of parents [h]. N = 7 × 5 larvae for both treatments. Kaplan-Meier estimated survival curves. (© Figure adapted from Capodeanu-Nägler *et al.* (2018))

Experiment 3: Effect of oral secretions on larval *N. orbicollis*

N. orbicollis larvae receiving baby mouse paste with oral secretions from parental beetles survived significantly longer than larvae that received plain baby mouse paste (log-rank test, $x^2 = 4.30$, $P = 0.038$, see **Fig. 2.4**). On average, larvae that received baby mouse paste without oral secretions survived 43.84 (± 3.39) hours, whereas larvae receiving baby mouse paste mixed with oral secretions survived 58.27 (± 6.37) hours on average. Five out of 35 larvae fed baby mouse paste with oral secretions pupated, but only one of 35 larvae fed plain baby mouse paste did.

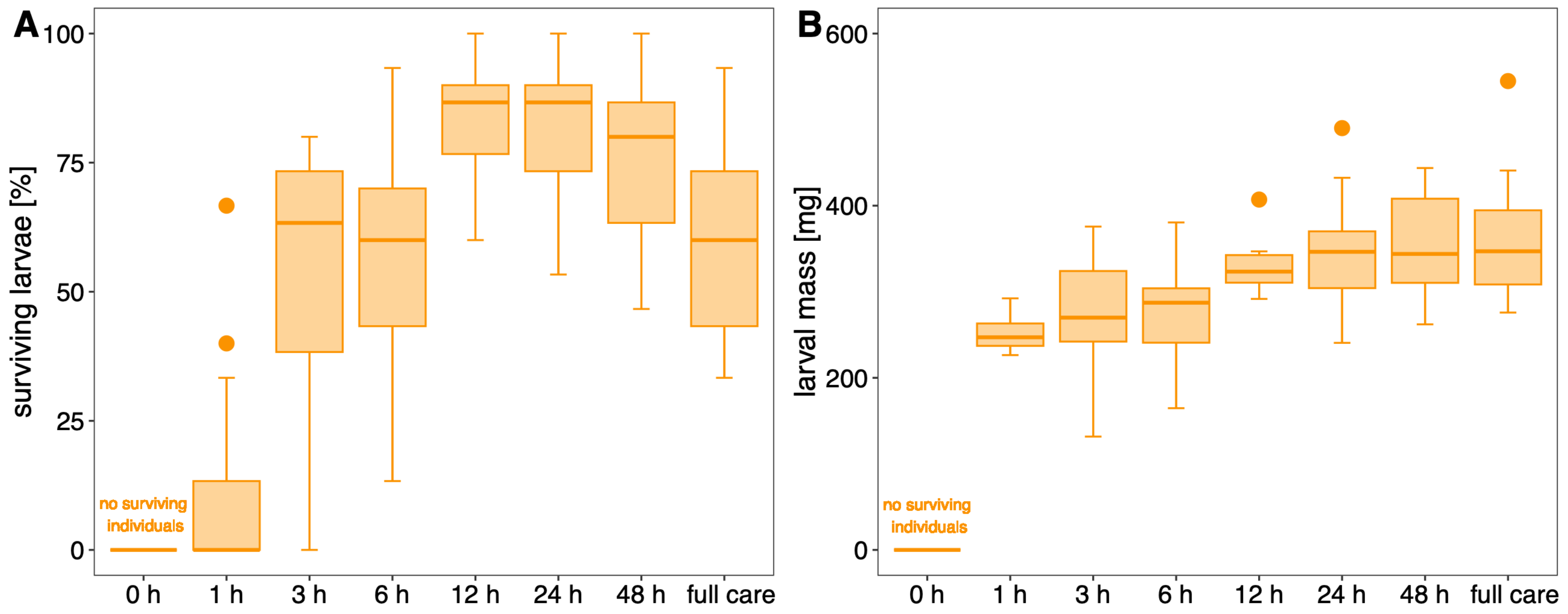

Figure 2.5
(A) Percent of larvae surviving to dispersal and (B) Mean larval mass per brood at dispersal [mg] of *N. orbicollis* larvae that received different durations of post-hatching care before parents were removed. N = 15 or 16 per treatment. Boxplots show median, interquartile range, minimum/maximum range. The dots are values that fall outside the interquartile range (> 1.5 × interquartile range). Different letters indicate significant differences between treatments. (© Figure adapted from Capodeanu-Nägler *et al.* (2018))

Experiment 4: Duration of post-hatching care needed to ensure independence in larval *N. orbicollis*

Out of a total of 1815 *N. orbicollis* larvae, 951 survived. Larval survival was significantly affected by the duration of post-hatching care that parents provided (GLM with Poisson errors: $F_{7,113} = 44.69$, $P < 0.001$, see **Fig. 2.5A**). We found that 3 h of care significantly increased the number of larvae that survived to dispersal compared to broods that received 0 h (Pairwise test: $P < 0.001$) or 1 h of care (Pairwise test: $P = 0.006$). When considering the number of broods in which some larvae survived, 1 h of care is not yet sufficient to significantly increase survival rate (broods with/without surviving larvae: 0 h: 0/15; 1 h: 4/ 11; Fisher's exact test: $P = 0.100$). However, it is clear that 3 h of care is sufficient to increase survival rate substantially; 87.5 % of the broods had surviving larvae after only 3 h of parental attendance compared to none with 0 h of care (broods with/without surviving larvae: 0 h: 0/15; 3 h: 14/2; Fisher's exact test: $P < 0.001$). In fact, 3 h of parental care did not differ from full care in terms of larval survival (Pairwise test: $P = 1.00$).

The duration of post-hatching care had a significant effect on larval mass (GLM with Gaussian errors: $F_{6,86} = 6.31$, $P < 0.001$, see **Fig. 2.5B**). The longer larvae received post-hatching care, the heavier they were when dispersing from the carcass. Within all surviving broods, larvae were significantly heavier when receiving 48 h of post-hatching care or full care than when receiving 3 h (Pairwise test: 48 h, $P = 0.003$; full care, $P = 0.002$) or 6 h of care (Pairwise test: 48 h, $P = 0.009$; full care, $P = 0.005$). As no larvae survived in the pre-hatching care treatment (0 h), we could not include this treatment in this analysis.

Discussion

The results of our study reveal new insights into the proximate causes of the extreme dependence of *N. orbicollis* offspring on parental care, and the variation in offspring dependence among species. We found that starvation tolerance of larvae varied among species, but did not appear to be related to dependence on parental care. Newly hatched *N. orbicollis* were generally able to self-feed, but the capacity for utilizing different types of food was more limited than in the more independent species, *N. pustulatus* and *N. vespilloides*. Dependent *N. orbicollis* gained less weight when self-feeding than nutritionally independent *N. pustulatus*. In addition, our study revealed that even a highly processed liquefied carrion meal is not sufficient to secure larval survival in *N. orbicollis*; however, oral secretions of parents mixed into a purée of baby mice prolong the survival of larval *N. orbicollis* without parents, but not long enough for most larvae to pupate. Finally, we revealed that three hours of post-hatching care was sufficient to achieve a significant increase in the survival and final mass of the larvae of the most dependent species, *N. orbicollis*. Our results highlight key characteristics of offspring and parental traits that augment our understanding of offspring dependence on parental care. Below, we elaborate on the wider implications of these results.

The results of our first experiment make it unlikely that starvation tolerance is related to high levels of offspring dependence, but is instead more likely associated with variation in growth rate. Here, we tested whether larvae of the three species, *N. orbicollis*, *N. pustulatus*, and *N. vespilloides*, differ in their tolerance to starvation in the absence of parents. Combined with information on egg investment, represented by mass at hatching (see **Fig. 2.3**), the level of starvation tolerance could provide information on whether larvae are fast or slow metabolisers, or on the parental investment in egg composition, and could thus be related to the marked offspring dependence on parental provisioning in *N. orbicollis*. As expected, we found that offspring of the more independent species, *N. pustulatus*, were most tolerant to starvation and survived the longest in the absence of food. Surprisingly, however, the highly dependent larvae of *N. orbicollis* starved to death later than larvae of *N. vespilloides*, which show an intermediate dependence on parental care (Capodeanu-Nägler *et al.* 2016). Here, hatchlings of *N. vespilloides* were the heaviest, followed by hatchlings of *N. orbicollis*, and then *N. pustulatus*, the lightest of the three species (but see Capodeanu-Nägler *et al.* 2017). Given their low mass at hatching, it is even more striking that most of the larval *N. pustulatus* were still alive when larvae of the other two species had all starved to death, suggesting that *N. pustulatus* are slow metabolisers. Generally, starvation resistance tends to increase with body size and larger energy stores, despite the greater absolute energy needs of larger individuals (Stockhoff 1991). However, larval *N. vespilloides* are not only the heaviest at hatching, but also have the highest growth rate of the three species during the first 48 h with full care (Capodeanu-Nägler *et al.* 2016). Faster growth rates are usually associated with a greater need for food and higher metabolic rates, making fast-growing individuals, such as *N. vespilloides*, more vulnerable to starvation when resources are limited (Blanckenhorn 2000).

The aim of our second experiment was to investigate whether hatchlings of *N. orbicollis* are able to self-feed, or whether traits necessary for self-feeding only develop at a later larval stage compared to the more independent species, which might explain strong offspring dependency on parental care. Larvae of passalid beetles, for example, differ in their ability to feed themselves and to construct feeding tunnels, and in their dependency on parental care (Tallamy & Wood 1986). Here, we found that newly hatched larvae of *N. orbicollis* were generally able to self-feed and gain weight when reared on baby mice, but not on prepared carcasses that parents usually use as a food resource for their offspring in nature. In contrast, no clear pattern was found in the two more independent species as larvae also increased in mass when provided with parent-prepared carcasses. It might not be surprising that larvae gain more weight on pieces of baby mice than, for example, on prepared carrion. First, baby mice are younger and probably have a higher water content, but fewer hard body parts than adult mice, making them more easily accessible for larvae. Second, pieces of baby mice are certainly fresher than the parent-prepared carrion. Further, the larvae of different species obviously differ in their ability to access and process different types of vertebrate carrion, which could be related to quantitative or qualitative differences in the oral digestive enzymes of larvae. It may be that the digestive system of young *N. orbicollis* hatchlings has evolved to rely more on pre-digested food from parents at the beginning, and later on, the slightly older larvae become able to consume and assimilate solid food on their own.

Alternatively, larval ability to self-feed might depend on species-specific characteristics of the mandibles. It is conceivable that mouthparts of *N. orbicollis* larvae may develop and sclerotize at a slower rate than the mouthparts of the other two species in our study, resulting in less robust mandibles that do not allow larvae to self-feed initially. Pukowski (Pukowski 1933) observed that hatchlings and recently moulted larvae of *N. vespillo* are unable to self-feed, and ascribed this to their unsclerotized mouthparts. Only after five to six hours, were larvae observed to self-feed (Pukowski 1933). Thus, differences in the sclerotization rate of mandibles could contribute to the variation in self-feeding and offspring dependence on parental care. In species with obligatory parental care, such as *N. orbicollis*, selection on mandible sclerotization rate or other traits, such as the production of digestive enzymes that could facilitate nutritional independence of offspring, may be relaxed as parents assume a greater share of the services related to food intake. As the expression and maintenance of these traits is generally costly (Lahti *et al.* 2009), traits related to self-feeding may only be expressed later in life when parents withdraw from providing parental care and offspring need to become independent. Generally, as soon as offspring traits are no longer in use because parents take over the tasks that secure offspring survival by providing parental care, a reduction in the relevant offspring traits is expected. This reduction, in turn, further drives the evolution of increased offspring dependency on parental care. For example, first instar neonates of wood-feeding *Cryptocercus* cockroaches, which exhibit elaborate biparental care, completely lack eyes and have a pale and thin cuticle (Nalepa *et al.* 2008). The hindgut symbionts that help larvae to metabolise and digest wood are not fully established until the third larval instar (Nalepa 1990). Consequently, until that time, nymphs depend on their parents for nutrition and symbiont transfer (Nalepa *et al.* 2008). Like *Cryptocercus*, first instar larvae of wood-feeding *Salganea* have a pale and transparent cuticle, and their eyes are present, but considerably reduced (Nalepa *et al.* 2008). Larvae feed on parental oral fluids and are somewhat less dependent than larval *Cryptocercus*, but more dependent than *Panesthia* neonates that are well developed and show no interactions with parents (Nalepa *et al.* 2008). In these three genera, the developmental characteristics of neonates appear to parallel a gradient of dependence on parental care (Nalepa *et al.* 2008).

Eggert, Reinking and Müller (1998) showed that 12 h of parental care resulted in a significant increase in survival and growth of larval *N. vespilloides*, suggesting that this was due, in part, to the opening in the carcass that is created by the parents, thereby facilitating easier access of the larvae to the carrion. In an experimental evolution study, larvae descended from beetles reared in the absence of post-hatching care became increasingly independent, a result that was attributed to the ability of larvae to self-feed more efficiently or through morphological adaptation of larval mouthparts (Schrader, Jarrett & Kilner 2015b). Although these behavioural or morphological adaptations are undoubtedly advantageous, their absence in larval *N. orbicollis* alone cannot explain their nutritional dependency. In our study, even an opening in the integument of a prepared carcass did not increase the efficiency of larvae to self-feed. Also, although larval *N. orbicollis* were able to consume small pieces of juvenile mouse carcasses, none of the larvae were able to survive more than 24 h in the absence of parents (A. Capodeanu-Nägler, pers. obs.). Even when provided with liquefied mouse carrion, most of the larvae did not survive to pupation.

One other factor that could account for the differences in self-feeding is the behaviour of larvae towards food when parents are absent. From a study on *N. vespilloides*, we know that larvae cooperate to penetrate the carcass when parents are absent (Schrader, Jarrett & Kilner 2015a). One precondition for cooperation between siblings is that larvae need to aggregate first. Generally, larvae seem to be attracted to one another and without another larva, larvae of the more independent species may have directly attempted to feed. However, larvae of *N. orbicollis* that benefit most from their parents' care, might be selected to focus on approaching their parents instead of converging to other larvae. Thus, especially when carcass preparation indicates the presence of parents by parent-derived cues on the carcass surface, larvae might wander around and search for a parent instead of attempting to feed (A. Capodeanu-Nägler, pers. obs.). Nevertheless, behavioural observations are needed to confirm these predictions.

Having shown that highly dependent *N. orbicollis* larvae are able to self-feed and increase in weight when provided with small pieces of baby mice, we attempted to determine whether they could be successfully reared in the absence of parents on a diet of homogenized mouse carrion mixed with oral secretions from parental beetles. We found that larvae reared on this diet were more likely to survive to dispersal than larvae receiving the same diet but without parental secretions. Thus, oral secretions of parents are clearly beneficial to *N. orbicollis* larvae, and may contain important symbionts, antimicrobial compounds, enzymes, or hormones. Eggert et al. (Eggert, Reinking & Müller 1998) examined the importance of symbiont transfer in *N. vespilloides*, but found that the positive effects of parental provisioning on larval survival and growth were not mediated by the transfer of symbionts. However, the transfer of symbionts in *N. orbicollis* may be more important as larvae in this species are more dependent on parental provisioning. In addition, the beetles' anal and oral secretions contain a wide range of compounds, some of which have antimicrobial properties (Degenkolb, Düring & Vilcinskas 2011; Hall *et al.* 2011; Arce, Smiseth & Rozen 2013), and express a variety of immune-related genes (Vogel, Badapanda & Vilcinskas 2011) with several antimicrobial peptides and lysozymes that are specifically upregulated in the presence of carrion (Jacobs *et al.* 2016; Palmer *et al.* 2016), and that could enhance offspring survival. Finally, parents may transfer growth-regulatory proteins or hormones that are essential for survival and development of dependent offspring. Juvenile hormone III (JH III), for example, has recently been found to be transferred to larvae by trophallaxis in ants (LeBoeuf 2017). In burying beetles, JH III plays a regulatory role in a multiple contexts (Trumbo, Borst & Robinson 1995; Trumbo 1997; Panaitof, Scott & Borst 2004; Scott & Panaitof 2004; Cotter & Kilner 2010b; Steiger *et al.* 2011; Engel *et al.* 2016), and parents might thus transfer some JH III when they regurgitate to larvae, which may contribute to their survival and growth (but see Crook, Flatt & Smiseth 2008).

Alternatively, oral secretions might signal the presence of parents to offspring. Carpenter ants, for instance, have been shown to exchange chemical signals by trophallaxis that help them to recognize nestmates (LeBoeuf *et al.* 2016; LeBoeuf 2017). Likewise, oral secretions of burying beetles might have a signalling function that helps larvae to localize pre-digested food or initiates larval feeding. Nonetheless, despite receiving homogenized carrion mixed with oral secretions of parents, most of the larvae of *N. orbicollis* did not survive until dispersal. However, since we do not know the actual volume of oral fluids that parents transfer to larvae, we may have provided larvae with less than the requisite amount of oral secretions. In our last experiment, we showed that larval survival and mass of *N. orbicollis* increased with the duration of post-hatching care, which is not surprising as parental care usually enhances offspring fitness (Clutton-Brock 1991; Balshine 2012). More surprisingly, we found that survival of the highly dependent *N. orbicollis* larvae was significantly enhanced after only three hours of parental care. Why might such a short period of care have such a profound effect on offspring survival? For *N. vespilloides*, larval begging as well as parental provisioning is known to peak 24 h after hatching (Smiseth, Darwell & Moore 2003; Smiseth, Lennox & Moore 2007). However, we observed larvae begging and parents provisioning in the first three hours after hatching (A. Capodeanu-Nägler and M. Prang, pers. obs.). Thus, parents might provide begging larvae with enough food during these first few hours that larvae have sufficient energy to survive until they are efficient self-feeders.

In the light of the other results of our study, however, we find it more likely that the transfer of oral secretions and maybe also anal secretions might be crucial for larval survival and growth, especially in the first few hours after larval hatching. For example, if larvae are given a single dose of symbionts by the parents in the first three hours after hatching, they may be able to survive thereafter. Burying beetles are known to harbour a diverse gut microbiome including various *Yarrowia*-like yeasts (Kaltenpoth & Steiger 2014). *Yarrowia* are present in both adult and larval life stages, and are possibly involved in carrion digestion and preservation (Vogel *et al.* 2017). More recent studies have shown that burying beetle parents not only transfer microorganisms to larvae via oral secretions, but tightly regulate the microbiome of the carcass by applying anal and oral secretions to it, which serves not only as a nutritional resource, but also facilitates the vertical transmission of symbiotic microbiota to larvae (Wang & Rozen 2017; Shukla, Vogel, *et al.* 2018; Wang & Rozen 2018). Thus, the transfer of preservation- and digestion-related microbiota to the carcass during the first hours might enhance larval survival for the more dependent offspring of *N. orbicollis* after parents have been removed.

Conclusions

Our study offers new insights into offspring and parental traits that appear to be relevant to the evolution of marked offspring dependence of certain species. We showed that tolerance to starvation differs greatly between species, but this is not likely to be associated with the high degree of offspring dependence in *N. orbicollis*. Nevertheless, *N. orbicollis* larvae are generally able to self-feed, but they are less efficient than larvae of the two more independent species. The variation in the efficiency to self-feed is probably not only due to differences in the structure or strength of larval mandibles, as larval *N. orbicollis* do not even survive when provided with liquefied mouse carrion for which the use of mandibles is redundant. As even short periods of parental care and easily accessible food containing oral secretions of parents significantly enhance survival of highly dependent *N. orbicollis*, we conclude that parental fluids must contain symbionts or other components that are crucial for larval survival. Thus, future studies should investigate the transfer and contents of oral fluids from parents to offspring more closely, which will further help to understand how coevolution drives an increasingly tight integration of offspring development and parental care (Badyaev & Uller 2009; Gardner & Smiseth 2011; Kölliker, Royle & Smiseth 2012; Uller 2012; Royle, Alonzo & Moore 2016; Kramer & Meunier 2017).

References

Anduaga, S. & Huerta, C. (2001) Effect of parental care on the duration of larval development and offspring survival in *Nicrophorus mexicanus* Matthews (Coleoptera: Silphidae). *The Coleopterists Bulletin,* 55, 264-271. https://doi.org/10.1649/0010-065X(2001)055[0264:EOPCOT]2.0.CO;2

Arce, A.N., Johnston, P.R., Smiseth, P.T. & Rozen, D.E. (2012) Mechanisms and fitness effects of antibacterial defences in a carrion beetle. *Journal of Evolutionary Biology,* 25, 930-937. https://doi.org/10.1111/j.1420-9101.2012.02486.x

Arce, A.N., Smiseth, P.T. & Rozen, D.E. (2013) Antimicrobial secretions and social immunity in larval burying beetles, *Nicrophorus vespilloides*. *Animal Behaviour,* 86, 741-745. https://doi.org/10.1016/j.anbehav.2013.07.008

Badyaev, A.V. & Uller, T. (2009) Parental effects in ecology and evolution: Mechanisms, processes and implications. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364, 1169-1177. https://doi.org/10.1098/rstb.2008.0302

Bairlein, F. (1999) Energy and nutrient utilization efficiencies in birds: A review. *Proceedings of the 22nd International Ornithological Congress, Durban. Bird-Life South Africa*, pp. 2221-2246. Durban, South Africa,

Ballard, O. & Morrow, A.L. (2013) Human milk composition: Nutrients and bioactive factors. *Pediatric clinics of North America,* 60, 49-74. https://doi.org/10.1016/j.pcl.2012.10.002

Balshine, S. (2012) Patterns of parental care in vertebrates. *The evolution of parental care* (eds N.J. Royle, P.T. Smiseth & M. Kölliker), pp. 62-80. Oxford Universtiy Press, Oxford, U.K., https://doi.org/10.1093/acprof:oso/9780199692576.003.0004

Behmer, S.T. (2009) Insect herbivore nutrient regulation. *Annual Review of Entomology,* 54, 165-187. https://doi.org/10.1146/annurev.ento.54.110807.090537

Blanckenhorn, W.U. (2000) The evolution of body size: What keeps organisms small? *The quarterly review of biology,* 75, 385-407. https://doi.org/10.1086/393620

Byrd, J.H. (2002) *Forensic entomology: The utility of arthropods in legal investigations forensic entomology.* CRC Press,

Capodeanu-Nägler, A., Eggert, A.-K., Vogel, H., Sakaluk, S.K. & Steiger, S. (2017) Species divergence in offspring begging and parental provisioning is linked to nutritional dependency. *Behavioral Ecology,* 29, 42-50. https://doi.org/10.1093/beheco/arx117

Capodeanu-Nägler, A., Keppner, E.M., Vogel, H., Ayasse, M., Eggert, A.-K., Sakaluk, S.K. & Steiger, S. (2016) From facultative to obligatory parental care: Interspecific variation in offspring dependency on post-hatching care in burying beetles. *Scientific Reports,* 6, 29323. https://doi.org/10.1038/srep29323

Capodeanu-Nägler, A., Prang, M.A., Trumbo, S.T., Vogel, H., Eggert, A.-K., Sakaluk, S.K. & Steiger, S. (2018) Offspring dependence on parental care and the role of parental transfer of oral fluids in burying beetles. *Frontiers in Zoology,* 15, 1-12. https://doi.org/10.1186/s12983-018-0278-5

Castro, G., Stoyan, N. & Myers, J.P. (1989) Assimilation efficiency in birds: A function of taxon or food type? *Comparative Biochemistry and Physiology Part A: Physiology,* 92A, 271-278. https://doi.org/10.1016/0300-9629(89)90563-X

Clutton-Brock, T.H. (1991) *The evolution of parental care*. Princeton University Press, Princeton, New Jersey, USA, https://doi.org/10.1046/j.1420-9101.1992.5040719.x

Costa, J.T. (2006) *The other insect societies*. Harvard University Press, Cambridge, Massachusetts, USA,

Cotter, S.C. & Kilner, R.M. (2010b) Sexual division of antibacterial resource defence in breeding burying beetles, *Nicrophorus vespilloides*. *Journal of Animal Ecology,* 79, 35-43. https://doi.org/10.1111/j.1365-2656.2009.01593.x

Crook, T.C., Flatt, T. & Smiseth, P.T. (2008) Hormonal modulation of larval begging and growth in the burying beetle *Nicrophorus vespilloides*. *Animal Behaviour,* 75, 71-77. https://doi.org/10.1016/j.anbehav.2007.04.009

Degenkolb, T., Düring, R.-A. & 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. https://doi.org/10.1007/s10886-011-9978-4

Eggert, A.-K. & Müller, J.K. (1997) Biparental care and social evolution in burying beetles: Lessons from the larder. *The evolution of social behavior in insects and arachnids* (eds J.C. Choe & B.J. Crespi), pp. 216-236. Cambridge, Massachusetts, U.S.A, https://doi.org/10.1017/CBO9780511721953.011

Eggert, A.-K., Reinking, M. & Müller, J.K. (1998) Parental care improves offspring survival and growth in burying beetles. *Animal Behaviour,* 55, 97-107. https://doi.org/10.1006/anbe.1997.0588

Engberg, R.M., Kaspers, B., Schranner, I., Kösters, J. & Lösch, U. (1992) Quantification of the immunoglobulin classes IgG and IgA in the young and adult pigeon (*Columba livia*). *Avian Pathology,* 21, 409-420. https://doi.org/10.1080/03079459208418859

Engel, K.C., Stökl, J., Schweizer, R., Vogel, H., Ayasse, M., Ruther, J. & Steiger, S. (2016) A hormone-related female anti-aphrodisiac signals temporary infertility and causes sexual abstinence to synchronize parental care. *Nature Communications,* 7, 10. https://doi.org/10.1038/ncomms11035

Eraud, C., Dorie, A., Jacquet, A. & Faivre, B. (2008) The crop milk: A potential new route for carotenoid-mediated parental effects. *J Avian Biol,* 39, 247-251. https://doi.org/10.1111/j.0908-8857.2008.04053.x

Fetherston, I.A., Scott, M.P. & Traniello, J.F.A. (1990) Parental care in burying beetles: The organization of male and female brood-care behavior. *Ethology,* 85, 177-190.

Gardner, A. & Smiseth, P.T. (2011) Evolution of parental care driven by mutual reinforcement of parental food provisioning and sibling competition. *Proceedings of the Royal Society B: Biological Sciences,* 278, 196-203. https://doi.org/10.1098/rspb.2010.1171

Hall, C.L., Wadsworth, N.K., Howard, D.R., Jennings, E.M., Farrell, L.D., Magnuson, T.S. & Smith, R.J. (2011) Inhibition of microorganisms on a carrion breeding resource: The antimicrobial peptide activity of burying beetle (Coleoptera: Silphidae) oral and anal secretions. *Environmental Entomology,* 40, 669-678. https://doi.org/10.1603/EN10137

Jacobs, C.G.C., Steiger, S., Heckel, D.G., Wielsch, N., Vilcinskas, A. & Vogel, H. (2016) Sex, offspring and carcass determine antimicrobial peptide expression in the burying beetle. *Scientific Reports,* 6, 25409. https://doi.org/10.1038/srep25409

Kaltenpoth, M. & Steiger, S. (2014) Unearthing carrion beetles' microbiome: Characterization of bacterial and fungal hindgut communities across the Silphidae. *Molecular Ecology,* 23, 1251-1267. https://doi.org/10.1111/mec.12469

Karasov, W.H. (1990) Digestion in birds: Chemical and physiological determinants and ecological implications. *Studies in avian biology,* 13, 391-415.

Kölliker, M., Royle, N.J. & Smiseth, P.T. (2012) Parent-offspring co-adaptation. *The evolution of parental care* (eds N.J. Royle, P.T. Smiseth & M. Kölliker), pp. 285-303. Oxford University Press, Oxford, U.K., https://doi.org/10.1093/acprof:oso/9780199692576.003.0016

Kramer, J. & Meunier, J. (2017) The evolution of social life in family groups. *bioRxiv*, 221192. https://doi.org/10.1101/221192

Lahti, D.C., Johnson, N.A., Ajie, B.C., Otto, S.P., Hendry, A.P., Blumstein, D.T., Coss, R.G., Donohue, K. & Foster, S.A. (2009) Relaxed selection in the wild. *Trends in Ecology & Evolution,* 24, 487-496. https://doi.org/10.1016/j.tree.2009.03.010

LeBoeuf, A.C. (2017) Trophallaxis. *Current Biology Magazine,* 27, R1299-R1300. https://doi.org/10.1016/j.cub.2017.10.047

LeBoeuf, A.C., Waridel, P., Brent, C.S., Gonçalves, A.N., Menin, L., Ortiz, D., Riba-Grognuz, O., Koto, A., Soares, Z.G., Privman, E., Miska, E.A., Benton, R. & Keller, L. (2016) Oral transfer of chemical cues, growth proteins and hormones in social insects. *Elife,* 5. https://doi.org/10.7554/eLife.20375

Maekawa, K., Matsumoto, T. & Nalepa, C.A. (2008) Social biology of the wood-feeding cockroach genus *Salganea* (Dictyoptera, Blaberidae, Panesthiinae): Did ovoviviparity prevent the evolution of eusociality in the lineage? *Insectes Sociaux,* 55, 107-114. https://doi.org/10.1007/s00040-008-0997-2

Müller, J.K. & Eggert, A.-K. (1990) Time-dependent shifts between infanticidal and parental behavior in female burying beetles: A mechanism of indirect mother-offspring recognition. *Behavioral Ecology and Sociobiology,* 27, 11-16. https://doi.org/10.1007/BF00183307

Nalepa, C.A. (1990) Early development of nymphs and establishment of hindgut symbiosis in *Cryptocercus punctulatus* (Dictyoptera: Cryptocercidae). *Annals of the Entomological Society of America,* 83, 786-789. https://doi.org/10.1093/aesa/83.4.786

Nalepa, C.A. & Bell, W.J. (1997) Postovulation parental investment and parental care in cockroaches. *The evolution of social behavior in insects and arachnids* (eds J.C. Choe & B.J. Crespi), pp. 26-51. Cambridge, Massachusetts, U.S.A,

Nalepa, C.A., Maekawa, K., Shimada, K., Saito, Y., Arellano, C. & Matsumoto, T. (2008) Altricial development in subsocial wood-feeding cockroaches. *Zoological Science,* 25, 1190-1198. https://doi.org/10.2108/zsj.25.1190

Palmer, W.J., Duarte, A., Schrader, M., Day, J.P., Kilner, R. & Jiggins, F.M. (2016) A gene associated with social immunity in the burying beetle *Nicrophorus vespilloides*. *Proceedings of the Royal Society B: Biological Sciences,* 283, 20152733. https://doi.org/10.1098/rspb.2015.2733

Panaitof, S.C., Scott, M.P. & Borst, D.W. (2004) Plasticity in juvenile hormone in male burying beetles during breeding: Physiological consequences of the loss of a mate. *Journal of Insect Physiology,* 50, 715-724. https://doi.org/10.1016/j.jinsphys.2004.05.008

Pukowski, E. (1933) Ökologische Untersuchungen an *Necrophorus* F. *Zeitschrift für Morphologie und Ökologie der Tiere,* 27, 518-586. https://doi.org/10.1007/BF00403155

Rauter, C.M. & Moore, A.J. (1999) Do honest signalling models of offspring solicitation apply to insects? *Proceedings of the Royal Society B: Biological Sciences,* 266, 1691. https://doi.org/10.1098/rspb.1999.0833

Rauter, C.M. & Moore, A.J. (2004) Time constraints and trade-offs among parental care behaviours: Effects of brood size, sex and loss of mate. *Animal Behaviour,* 68, 695-702. https://doi.org/10.1016/j.anbehav.2003.09.018

Royle, N.J., Alonzo, S.H. & Moore, A.J. (2016) Co-evolution, conflict and complexity: What have we learned about the evolution of parental care behaviours? *Current opinion in behavioral sciences,* 12, 30-36. https://doi.org/10.1016/j.cobeha.2016.08.004

Royle, N.J., Russell, A.F. & Wilson, A.J. (2014) The evolution of flexible parenting. *Science,* 345, 776-781. https://doi.org/10.1126/science.1253294

Satou, A., Nisimura, T. & Numata, H. (2001) Cost and necessity of parental care in the burying beetle *Nicrophorus quadripunctatus*. *Zoological Science,* 18, 975-979. https://doi.org/10.2108/zsj.18.975

Schrader, M., Jarrett, B.J. & Kilner, R.M. (2015a) Parental care masks a density-dependent shift from cooperation to competition among burying beetle larvae. *Evolution,* 69, 1077-1084. https://doi.org/10.1111/evo.12615

Schrader, M., Jarrett, B.J. & Kilner, R.M. (2015b) Using experimental evolution to study adaptations for life within the family. *The American Naturalist,* 185, 610-619. https://doi.org/10.1086/680500

Scott, M.P. (1998) The ecology and behavior of burying beetles. *Annual Review of Entomology,* 43, 595-618. https://doi.org/10.1146/annurev.ento.43.1.595

Scott, M.P. & Panaitof, S.C. (2004) Social stimuli affect juvenile hormone during breeding in biparental burying beetles (Silphidae: *Nicrophorus*). *Hormones and Behavior,* 45, 159-167. https://doi.org/10.1016/j.yhbeh.2003.09.012

Scott, M.P. & Traniello, J.F.A. (1990) Behavioural and ecological correlates of male and female parental care and reproductive success in burying beetles (*Nicrophorus* spp.). *Animal Behaviour,* 39, 274-283. https://doi.org/10.1016/S0003-3472(05)80871-1

Scriber, J.M. & Slansky, F. (1981) The nutritional ecology of immature insects. *Annual Review of Entomology,* 26, 183-211. https://doi.org/10.1146/annurev.en.26.010181.001151

Shetty, S., Bharathi, L., Shenoy, K.B. & Hegde, S.N. (1992) Biochemical properties of pigeon milk and its effect on growth. *J Comp Physiol B,* 162, 632-636. https://doi.org/10.1007/bf00296644

Shimada, K. & Maekawa, K. (2011) Description of the basic features of parent-offspring stomodeal trophallaxis in the subsocial wood-feeding cockroach *Salganea esakii* (Dictyoptera, Blaberidae, Panesthiinae). *Entomological Science,* 14, 9-12. https://doi.org/10.1111/j.1479-8298.2010.00406.x

Shukla, S.P., Vogel, H., Heckel, D.G., Vilcinskas, A. & Kaltenpoth, M. (2018) Burying beetles regulate the microbiome of carcasses and use it to transmit a core microbiota to their offspring. *Molecular Ecology,* 27, 1980-1991. https://doi.org/10.1111/mec.14269

Sikes, D.S. & Venables, C. (2013) Molecular phylogeny of the burying beetles (Coleoptera: Silphidae: Nicrophorinae). *Molecular Phylogenetics and Evolution,* 69, 552-565. https://doi.org/10.1016/j.ympev.2013.07.022

Slansky, F. (1982) Insect nutrition: An adaptationist's perspective. *Fla Entomol,* 65, 45-71.

Smiseth, P.T., Darwell, C.T. & Moore, A.J. (2003) Partial begging: An empirical model for the early evolution of offspring signalling. *Proceedings of the Royal Society B: Biological Sciences,* 270, 1773-1777. https://doi.org/10.1098/rspb.2003.2444

Smiseth, P.T., Lennox, L. & Moore, A.J. (2007) Interaction between parental care and sibling competition: Parents enhance offspring growth and exacerbate sibling competition. *Evolution,* 61, 2331-2339. https://doi.org/10.1111/j.1558-5646.2007.00192.x

Smiseth, P.T. & Moore, A.J. (2002) Does resource availability affect offspring begging and parental provisioning in a partially begging species? *Animal Behaviour,* 63, 577-585. https://doi.org/10.1006/anbe.2001.1944

Steiger, S., Gershman, S.N., Pettinger, A.M., Eggert, A.-K. & 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. https://doi.org/10.1111/j.1365-2435.2011.01895.x

Stockhoff, B.A. (1991) Starvation resistance of gypsy moth, *Lymantria dispar* (L.)(Lepidoptera: Lymantriidae): Tradeoffs among growth, body size, and survival. *Oecologia,* 88, 422-429.

Tallamy, D.W. & Wood, T.K. (1986) Convergence patterns in subsocial insects. *Annual Review of Entomology,* 31, 369-390.

Trumbo, S.T. (1992) Monogamy to communal breeding: Exploitation of a broad resource base by burying beetles (*Nicrophorus*). *Ecological Entomology,* 17, 289-298. https://doi.org/10.1111/j.1365-2311.1992.tb01060.x

Trumbo, S.T. (1997) Juvenile hormone-mediated reproduction in burying beetles: From behavior to physiology. *Archives of Insect Biochemistry and Physiology,* 35, 479-490. https://doi.org/10.1002/(Sici)1520-6327(1997)35:4<479::Aid-Arch9>3.3.Co;2-3

Trumbo, S.T., Borst, D.W. & Robinson, G.E. (1995) Rapid elevation of juvenile-hormone titer during behavioral assessment of the breeding resource by the burying beetle, *Nicrophorus orbicollis*. *Journal of Insect Physiology,* 41, 535-543. https://doi.org/10.1016/0022-1910(94)00127-3

Uller, T. (2012) Parental effects in development and evolution. *The evolution of parental care* (eds N.J. Royle, P.T. Smiseth & M. Kölliker), pp. 246-266. Oxford University Press, Oxford, U.K., https://doi.org/10.1093/acprof:oso/9780199692576.003.0014

Vogel, H., Badapanda, C. & Vilcinskas, A. (2011) Identification of immunity-related genes in the burying beetle *Nicrophorus vespilloides* by suppression subtractive hybridization. *Insect Molecular Biology,* 20, 787-800. https://doi.org/10.1111/j.1365-2583.2011.01109.x

Vogel, H., Shukla, S.P., Engl, T., Weiss, B., Fischer, R., Steiger, S., Heckel, D.G., Kaltenpoth, M. & Vilcinskas, A. (2017) The digestive and defensive basis of carcass utilization by the burying beetle and its microbiota. *Nature Communications,* 8, 1-15. https://doi.org/10.1038/ncomms15186

Wang, Y. & Rozen, D.E. (2017) Gut microbiota colonization and transmission in the burying beetle *Nicrophorus vespilloides* throughout development. *Applied and Environmental Microbiology,* 83, e03250-03216. https://doi.org/10.1128/aem.03250-16

Wang, Y. & Rozen, D.E. (2018) Gut microbiota in the burying beetle, *Nicrophorus vespilloides*, provide colonization resistance against larval bacterial pathogens. *Ecology and Evolution,* 8, 1646-1654. https://doi.org/10.1002/ece3.3589

Wong, J.W.Y., Meunier, J. & Kölliker, M. (2013) The evolution of parental care in insects: The roles of ecology, life history and the social environment. *Ecological Entomology,* 38, 123-137. https://doi.org/10.1111/een.12000

Publication 3: From nutritious nests to parental provisioning: Unveiling the intricate balance of direct and indirect parental care in a highly dependent system

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Ready to submit

M.A.P. and S.S. conceived and designed the study. M.A.P., D.L., and P.S. performed the experiments. M.A.P analyzed the data. M.A.P and S.S. discussed the results. M.A.P wrote the first draft of the manuscript; all authors revised it until completion.

Own contribution: Concept and study design 50 %, data acquisition 40 %, data analyses and figures 95 %, interpretation of results 50 %, manuscript writing 70 %.

Abstract

Parental care has evolved several times in the animal kingdom and includes all parental traits that increase the fitness of the offspring. The evolution of care can lead to prolonged association of parents with their offspring. This, in turn, can lead to positive evolutionary feedback loops that not only create systems in which multiple care behaviors are exhibited, but also allow offspring to become more dependent on parental care. The most common forms of parental care are often indirect, such as selecting a suitable oviposition site or building a nest. Direct care behaviors, such as providing food, are less common and usually more costly. However, the extent to which offspring rely on indirect versus direct care is often unknown. In this study, we aim to elucidate the role and importance of direct and indirect care in a system where offspring are highly dependent on parental care. We conducted an experiment in which we manipulated the duration and composition of direct and indirect care using the burying beetle *Nicrophorus orbicollis*. Burying beetles reproduce by exploiting small vertebrate carcasses, which they bury and convert into a nutritious nursery for their offspring. After hatching, the larvae are protected from predators within the carcass. In addition to modifying the food resource by creating a feeding cavity, an example of indirect care, parents actively feed their offspring, providing direct care. We found that direct care had a greater effect on offspring growth and survival than indirect care, but that indirect care also improved offspring fitness, especially when parents abandoned the offspring prematurely.

Key words

Carcass breeders, direct and indirect care, *Nicrophorus,* offspring dependence, parental care.

Introduction

Parental care has evolved multiple times in the animal kingdom whenever its benefits outweigh the costs it imposes on parents. Parental care is defined as ‘any parental trait that enhances the fitness of a parent’s offspring, and that is likely to have originated and/or to be currently maintained for this function (Clutton-Brock 1991; Smiseth, Kölliker & Royle 2012). The most common forms of parental care are indirect. Indirect parental care is defined as parental traits that do not require physical contact with offspring (e.g., the construction of a nest or the selection of a suitable oviposition site; Kleiman & Malcolm 1981). As such, the beneficial effect of indirect care could be masked by the more elaborate forms of parental care (vivipary and food provisioning; see e.g., Kramer & Meunier 2019) especially if the offspring is obligate dependent on parental care.

Although indirect care behaviors seem to be less valuable, they are suggested to be important or in some species even essential for offspring survival (Clutton-Brock 1991). Nest building and selection of a suitable oviposition site protects the offspring against adverse biotic environmental conditions, like predators, but it also shields them from harmful/detrimental abiotic environmental conditions, like flooding, desiccation, or extreme temperatures (Smiseth, Kölliker & Royle 2012; Meunier, Körner & Kramer 2022). Indirect forms of parental care can vary highly within the animal kingdom, especially nest building can range from the basic burial of eggs in the substrate (Baur 1994) to the construction of highly elaborate nests (Winkler & Sheldon 1993; Grubbauer & Hoi 1996). Sometimes the nest provides even immunological benefits, e.g., the incorporation of antimicrobial plant properties in blue tit *Cyanistes caeruleus* nests (Mennerat *et al.* 2009) or acts as a microbial source and filter (Ruiz-Castellano *et al.* 2016; Campos-Cerda & Bohannan 2020), so that only beneficial bacteria can establish within the nest environment, e.g., the incorporation of beneficial bacteria in the beewolf *Philanthus triangulum* (Kaltenpoth *et al.* 2005).

In contrast to this, direct forms of parental care are defined as parental traits that require physical contact between parents and offspring. e.g., by brooding the eggs and/or the offspring, or by progressively providing food to the offspring (Kleiman & Malcolm 1981). When parents provide their offspring directly with food via regurgitation (trophallaxis) or milk producing glands, they sometimes add beneficial components, such as food predigesting enzymes, microbial symbionts, or immunity-related components. Mammalian milk, for example, contains antimicrobial agents, anti-inflammatory factors and immunomodulators (Goldman 1993; Caccavo *et al.* 2002; Milani *et al.* 2017) and doves, feed their chicks with crop milk containing beneficial carotenoids (Eraud *et al.* 2008).

Generally, the evolution of parental care is thought to be very dynamic, involving the rapid coevolution of parental and filial traits (Gardner & Smiseth 2011; Smiseth, Kölliker & Royle 2012). Components of care are not only target of selection but can shape other care traits (Duarte et al 2021) as well as offspring phenotypes, as it creates microhabitats to which offspring adapt (Gardner & Smiseth 2011; Smiseth, Kölliker & Royle 2012; Socias-Martínez & Kappeler 2019). The result can be positive evolutionary feedback loops that not only allow offspring to become more dependent on parental care, but also can increase care and give rise to systems in which multiple care behaviours are exhibited (Gardner & Smiseth 2011; Trumbo 2012; Kramer & Meunier 2019). Hence, the resulting care strategies can be inextricably complex comprising multiple traits that increase offspring survival, growth and/or quality and ultimately offspring lifetime reproductive success. Most studies only focus on the effect of parental presence on offspring fitness by removing the parent. Generally, this leads to a decline in offspring fitness. However, sometimes, parental care is more complex and if composed of different parts, this approach usually does not clarify how important different components of care are for offspring fitness.

Burying beetles are an ideal model system to better understand fitness consequences of different components and the amount of care, because they show a high variation in offspring dependence on parental care between species and aspects of care can be readily manipulated (Capodeanu-Nägler *et al.* 2016). Species of this genus reproduce on small vertebrate carcasses (Pukowski 1933; Eggert & Müller 1997). During pre-hatching care both parents bury the carcass and convert it into an edible nursery by removing fur or feathers, treating the carcass with oral and anal antimicrobial secretions, and inoculating the carcass with symbiotic microbiota (Pukowski 1933; Hoback *et al.* 2004; Cotter & Kilner 2010a; Hall *et al.* 2011; Vogel *et al.* 2017; Shukla, Plata*, et al.* 2018; Shukla, Vogel*, et al.* 2018; Miller *et al.* 2019).

During post-hatching care, the offspring are both directly provided with regurgitated food (Pukowski 1933; Pukowski 1934a; Milne & Milne 1976) and indirectly cared for by the parents, as parents protect the carcass and the offspring from predators, competitors, and conspecifics and maintain and modify the nest. The parents also chew a hole into the carcass (hereafter referred to as feeding cavity) within which the larvae aggregate (Trumbo 1992; Eggert, Reinking & Müller 1998; Capodeanu-Nägler *et al.* 2016). The parental secretions act predigestive and inoculate the feeding cavity with beneficial microbes (Hall *et al.* 2011; Shukla, Plata*, et al.* 2018; Shukla, Vogel, *et al.* 2018) which facilitate larval self-feeding (Trumbo 1992; Eggert, Reinking & Müller 1998; Capodeanu-Nägler *et al.* 2016; Shukla, Plata*, et al.* 2018). In *N. vespilloides*, these microbes were shown to include the yeast *Yarrowia* which are transferred via anal secretions by the parental beetles (Kaltenpoth & Steiger 2014; Vogel *et al.* 2017; Shukla, Plata*, et al.* 2018) and thus are vertically transmitted via the carcass surface to their offspring. *N. vespilloides* larvae that develop in unmodified nests lack these beneficial microbes, and instead seem to acquire less beneficial carcass-borne microbes (Wang & Rozen 2018).

Although all species of this genus modify the nest and provide larvae with regurgitated food, these species' offspring vary dramatically in their dependence on parental care (Trumbo 1992; Capodeanu-Nägler *et al.* 2016). Some species like *N. pustulatus* are largely independent from parental care, whereas in *N. orbicollis,* offspring rarely survive in absence of parents (Trumbo 1992; Capodeanu-Nägler *et al.* 2016). Previous studies show that offspring do not seem to rely on the modification of the nest in the pre-hatching phase (Trumbo 1992; Capodeanu-Nägler *et al.* 2016) but rather on components of post-hatching care. Especially parental oral secretions which are part of both, the direct care (food provisioning via oral trophallaxis) and indirect care (nest modification and manipulation of the feeding cavity) seem to play an important role, as the addition of parental oral secretions to a mixture of pureed carrion food could increase larval survival in the highly dependent *N. orbicollis* (Capodeanu-Nägler *et al.* 2018). In the intermediate dependent species *N. vespilloides,* Eggert, Reinking and Müller (1998) demonstrated that parental post-hatching care positively influences offspring fitness. A key indicator for understanding the proximate causes of offspring dependence appears to be the duration of parental care. Capodeanu-Nägler et al. (2018) discovered that a mere 3 hours of post-hatching care significantly boosts larval survival. Extending this care to 12 hours resulted in larval performance that was virtually indistinguishable from that observed with the full duration of care, as larvae typically disperse for pupation around the 120-hour mark. Consequently, a brief period of parental care is sufficient to secure larval survival. Nonetheless, it remains uncertain whether this enhanced survival is primarily attributed to the direct transfer of oral secretions through regurgitation, or to the manipulation of the feeding cavity by the parents.

In this study we aim to shed light on the importance of direct and indirect post-hatching care by conducting an experiment with the highly dependent burying beetle species *N. orbicollis*, in which we manipulated the duration of the presence of direct (food provisioning via oral trophallaxis) and indirect post-hatching care (nest modification and manipulation of the feeding cavity). To this end, we subjected larvae to four different treatments. In the 'direct care' treatment, larvae had access to parents (direct care) before they were relocated to a parentally unmodified nest environment (no indirect care). In the 'indirect care' treatment, larvae were not allowed to have contact to parents (no direct care) but were placed on a nest that had been allowed to be modified by the parents in the pre- and post-hatching phase (indirect care). In the third and fourth treatment, larvae received access to parents as well as to a parentally modified nest (direct + indirect care). However, as larvae of the third group remained in their native nest, larvae of the fourth group were relocated to a foreign nest to control for a potential effect of larval relocation. Direct and/or indirect post-hatching care was either provided for 3 or 12 h. To shed light on the fitness consequences, we measured the growth and survival rate of offspring. We predicted that (1) larvae would perform better with an increase in the duration of post-hatching care and (2) larvae receiving both types of care would perform better than those that received only indirect care or those that received direct care but were relocated to an unmodified nest environment. We had no a priori prediction for whether direct or indirect care was more important for larval fitness.

Methods

Origin and husbandry of beetles

Experimental *Nicrophorus orbicollis* were descendants (6th and 8th generation) from beetles caught from carrion-baited pitfall traps near Big Falls, Wisconsin, USA (44°36'59.0' N, 89°00'58.0' W). Beetles were kept in groups of up to five same-sex siblings in boxes (10 x 10 x 6 cm) filled to two thirds with moist peat and kept in a 16:8 L:D cycle at 20 °C. All beetles were fed with cut mealworms (*Tenebrio molitor* and *Zophobas morio*) *ad libitum* twice a week.

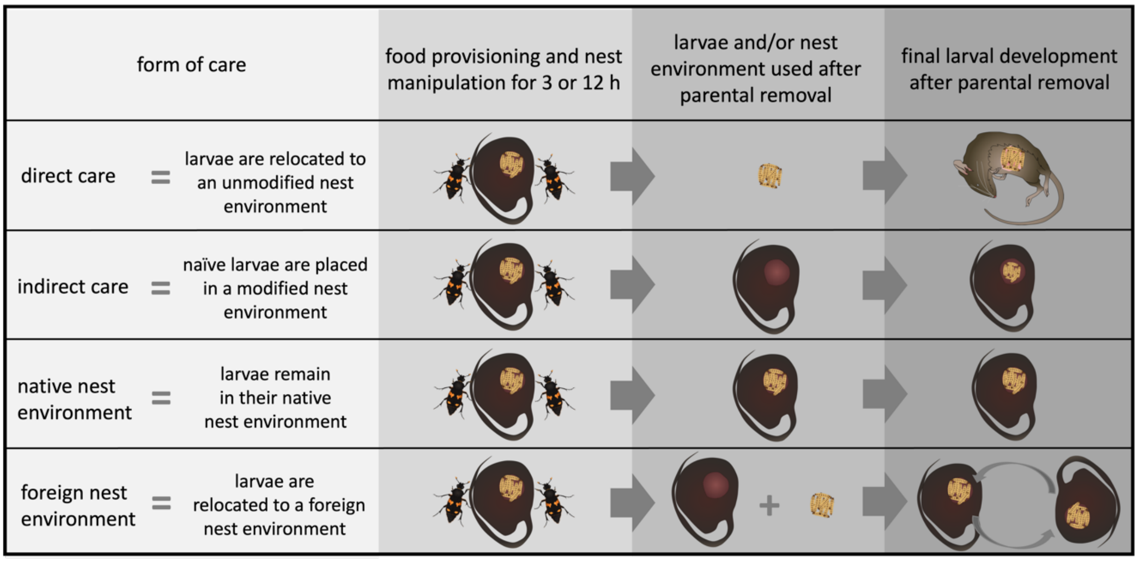

Figure 3.1
The design of the experiment. The three panels display the different steps of the experiment. Larvae received either 3 or 12 h of parental care, before parents were removed. Thereafter the groups were split in four parts: 'direct care', 'indirect care', 'native nest environment' and 'foreign nest environment'.

Experimental design and procedures

Reproduction was induced by providing non-sibling virgin males and females that were paired haphazardly with a thawed mouse carcass of 17.5 – 21.5 g (Frostfutter.de – B.A.F. Group GmbH, Germany). We used around 200 pairs to generate larvae and carcasses for the experiment. The pairs and their mouse carcass were put into plastic boxes (10 x 10 x 6 cm) filled to one third with moist peat and placed in a dark climate chamber at 20 °C. 24 h before larvae were expected to hatch (expected start of hatching in *N. orbicollis*: 96 h), the parents and their mouse carcass were placed in a new peat-filled box (10 x 10 x 6 cm) to prevent contact between parents and hatched larvae. The old boxes containing the eggs were checked several times a day (at least every 4 h day and night) for larval hatching. Newly hatched larvae were pooled to control for within-family variation and individual differences and placed in a Petri dish with moist filter paper at 4 °C until they were assigned to a treatment. To investigate the importance of indirect and direct aspects of post-hatching care for larval growth and survival, we standardized the brood size. For this, we haphazardly chose 10 larvae out of the Petri dish and assigned them to parents, whose own larvae already hatched to avoid the time-dependent infanticide (Müller & Eggert 1990). Larvae then received full parental post-hatching care for either 3 or 12 h. Thereafter parents were removed, and the parentally nourished larvae and/or their modified carcasses were assigned to one of the four following treatments (**Fig. 3.1**). In the 'native nest environment' treatment (3 h and 12 h each n = 18), the parentally nourished larvae remained on their original carcass as a control treatment. Parentally nourished larvae of the 'foreign nest environment' (3 h: n = 16; 12 h: n = 17) were relocated from one modified carcass to another within their treatment group to control for the relocation of larvae to another carcass. Parentally nourished larvae of the 'direct care' treatment (3 h: n = 18; 12 h: n = 17) were relocated to a parentally unmodified nest environment (fresh mouse carcass with an artificially cut 1 cm wide feeding cavity to allow larvae access to the flesh). For the 'indirect care' treatment (3 h: n = 18; 12 h: n = 17) freshly hatched larvae, which had no contact to parents and therefore did not obtain any direct care were placed in a parentally modified nest environment (i.e., the mouse carcasses from the 'direct care' group, on which 10 larvae were previously provided with both aspects of post-hatching care for either 3 or 12 h).

Larval mass was measured at hatching (0 h) and in the treatments 'native nest environment', 'foreign nest environment', and 'direct care' the surviving larvae were counted when parents were removed at 3 h or 12 h. Thereafter, we weighted and counted the surviving larvae of all treatment groups when larvae were 24 h old and at the time of larval dispersal (approximately 120 h), when larvae were visibly leaving the remains of the carcass for pupation. We decided on using 24 h as time point, because previous studies showed that *N. orbicollis* often die after this time in the total absence of post-hatching care (Trumbo 1992; Capodeanu-Nägler *et al.* 2016). Larvae were first gently removed from the feeding cavity, counted, weighted, and thereafter returned to their mouse carcass.

Statistics

All data were analyzed and plotted using R (version 4.1.0, R Core team 2014) loaded with the packages ‘car_3.0-9’, ‘cowplot_1.0.0’, ‘emmeans_1.4.8’, ‘ggnewscale_0.4.3’, ggplot2_3.3.2’, ’multcomp_1.4-10’, ‘multcompView_0.1-8’, ‘tidyr_1.1.0’. Larval performance was measured using two parameters: growth rate and survival ratio. The larval survival ratios at 24 h and at dispersal were calculated by dividing the number of surviving larvae at 24 h (or dispersal) by 10. The larval growth rates were calculated using the formula $GR = \frac{lm_X - lm_0}{lm_0}$, where lm_X is the mean larval mass of a brood at 24 h or larval dispersal and lm_0 the mean larval mass of a brood at hatching (as described in Prang *et al.* 2022).

We performed generalized linear models (GLMs) with the duration of care and the type of care larvae received and their interaction as fixed factor. We applied GLMs with a gaussian error structure to test for effects on larval growth rate and GLMs with a quasi-binomial error structure to test for the effects on larval survival ratio (all with a logit link function). For larval growth rate, we excluded treatment groups where only one brood had surviving larvae from further analyses. At 24 h this included larvae from the group ‘indirect care’ with 3 h of post-hatching care and at dispersal larvae from both ‘indirect care’ groups and the larvae from the ‘direct care’ group with 3 h of post-hatching care. We obtained *p*-values for the general effects by using the ‘Anova’ function with type ‘III’ sum of squares of the ‘car’ package.

We analyzed within-group differences using the function emmeans() of the package ‘emmeans’. As larval growth rate at dispersal was significantly influenced by the interaction between duration of care and type of care, we analyzed the within-group differences using the post hoc comparison of the function emmeans(). In all cases, the p-values were adjusted using the Holm-Bonferroni method.

Results

Table 3.1

Summary of models for the effects of the duration of post-hatching care (3 h or 12 h) and type of care ('direct care', 'indirect care, 'native nest environment' and 'foreign nest environment') and their interactions on larval survival ratio and growth rate in the first 24 h after hatching. Larval survival ratio and growth rate at 24 h were analyzed separately. Significant values are in bold.

predictors	survival ratio				growth rate			
	Sum Sq	df	F	*P*-value	Sum Sq	df	F	*P*-value
duration of care	1.48	1	5.14	**0.0025**	19.87	1	73.01	**< 0.001**
larval treatment	55.83	3	64.77	**< 0.001**	3.62	2	6.64	**0.0020**
duration of care * larval treatment	1.46	3	1.70	0.17	0.25	2	0.46	0.063
residuals	37.64	131			26.13	96		

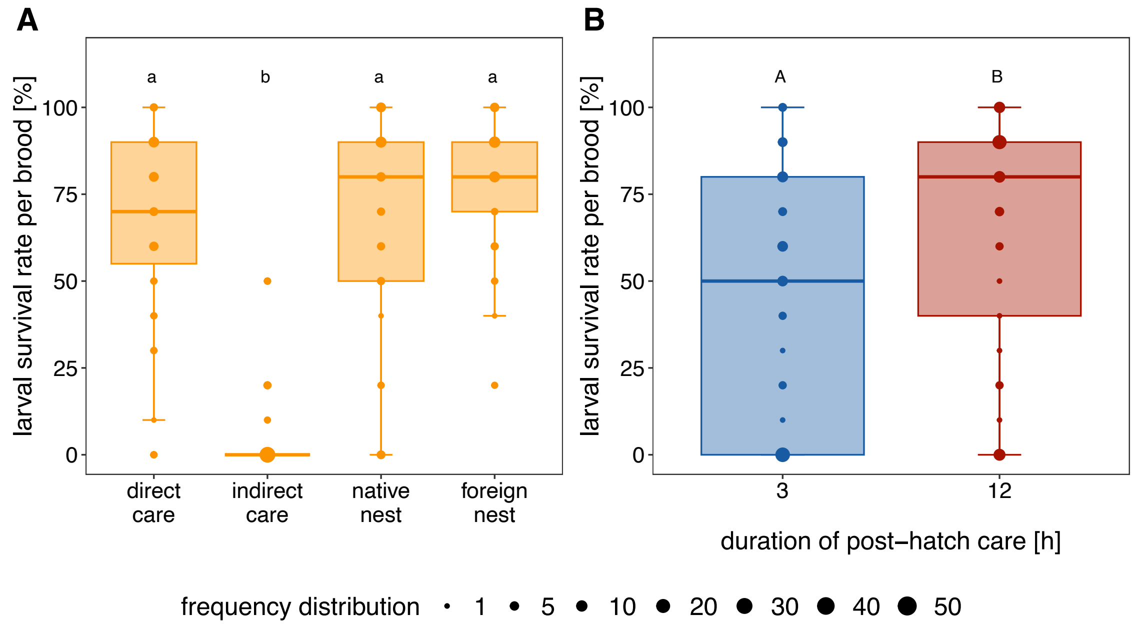

Figure 3.2
Larval survival ratio at 24 h after hatching in relation to (A) type of care and (B) duration of care. Larvae received either 3 h or 12 h of post-hatching care and one of four types of parental care: 'direct care' (3 h: n = 18; 12 h: n = 17), 'indirect care' (3 h: n = 18; 12 h: n = 17), 'native nest environment' (3 h: n = 16; 12 h: n = 18), or 'foreign nest environment' (3 h: n = 16; 12 h: n = 17). Boxplots show median and the interquartile range and the data point size equals the number of broods with the same survival. Different letters indicate significant differences among the four levels of (A) types of care or between the two levels of (B) duration of post-hatch care.

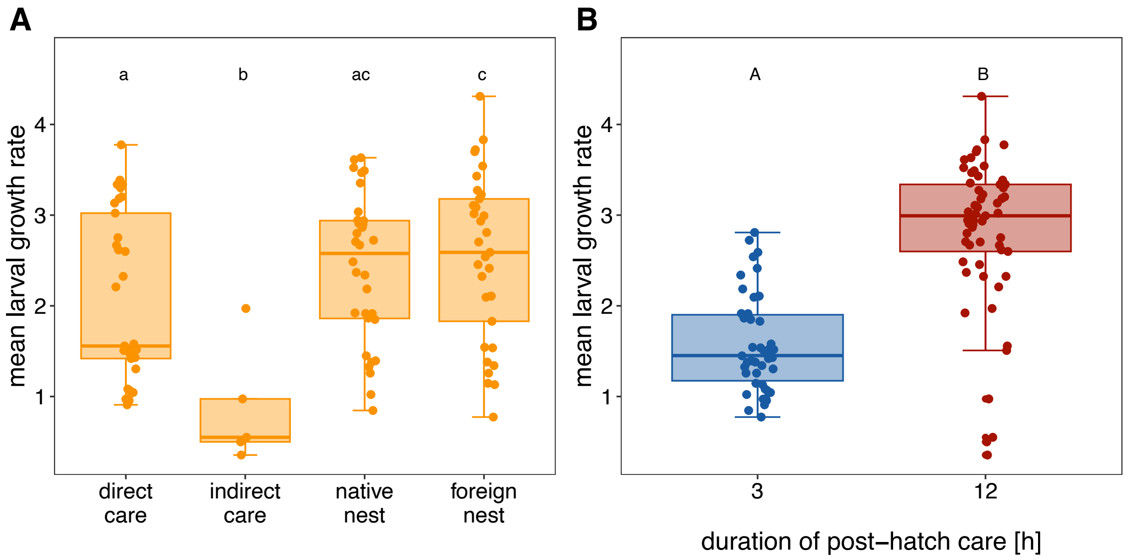

Figure 3.3
Mean growth rate of the surviving larvae at 24 h after hatching in relation to (A) type of care and (B) duration of care. Larvae received either 3 h or 12 h of post-hatching care and one of four types of parental care: 'direct care' (3 h: n = 16; 12 h: n = 17), 'indirect care' (3 h: n = 1; 12 h: n = 5), 'native nest environment' (3 h: 14; 12 h: n = 18), or 'foreign nest environment' (3 h: n = 16; 12 h: n = 17). Boxplots show median and the interquartile range and the data point size equals the number of broods with the same survival. Different letters indicate significant differences among the four levels of (A) types of care or between the two levels of (B) duration of post-hatch care. Groups with one single value or less were excluded from these analyses.

Larval performance at 24 h

At 24 h, larval survival ratio and growth rate were influenced by the duration of post-hatching care and by the type of care, but not by their interaction (**Table 3.1**). Larval performance differed among the types of care larvae received (**Fig. 3.2A**, **Fig. 3.3A**) and larvae grew faster and survived more likely when the duration of post-hatching care was increased (**Fig. 3.2B, Fig. 3.3B**).

Post-hoc comparisons revealed that that the relocation of larvae did not affect their survival ratio or growth rate, as larvae that received full care survived and grew equally well in their native nest environment and the foreign nest environment (**Fig. 3.2A, Fig. 3.3A**). Although larvae that received 'direct care' survived as well as the treatments that received both type of care (**Fig. 3.2A**, 'native nest environment', 'foreign nest environment'), they grew slower than larvae that were relocated to a 'foreign nest environment' (**Fig. 3.3A**). Larvae receiving only 'indirect care' survived worse and grew at the slowest rate compared to larvae receiving other types of care (**Fig. 3.2A, Fig. 3.3A**). Note that only larvae survived that received 12 h of 'indirect care', their counterparts with 3 h of 'indirect care' did not (**Fig. 3.2A**).

Table 3.2
Summary of models for the effects of the duration of post-hatching care (3 h or 12 h) and type of care (‘direct care’, ‘indirect care, ‘native nest environment’ and ‘foreign nest environment’) and their interactions on larval survival ratio and growth rate at dispersal. Larval survival ratio and growth rate at dispersal were analyzed separately. Significant values are in bold.

predictors	survival ratio				growth rate			
	Sum Sq	df	F	*P*-value	Sum Sq	df	F	*P*-value
duration of care	7.57	1	24.94	**< 0.001**	37	1	0.074	0.79
larval treatment	43.13	3	47.39	**< 0.001**	16	1	0.031	0.86
duration of care * larval treatment	1.88	3	2.07	0.11	2421	1	4.82	**0.032**
residuals	39.75	131			32674	65		

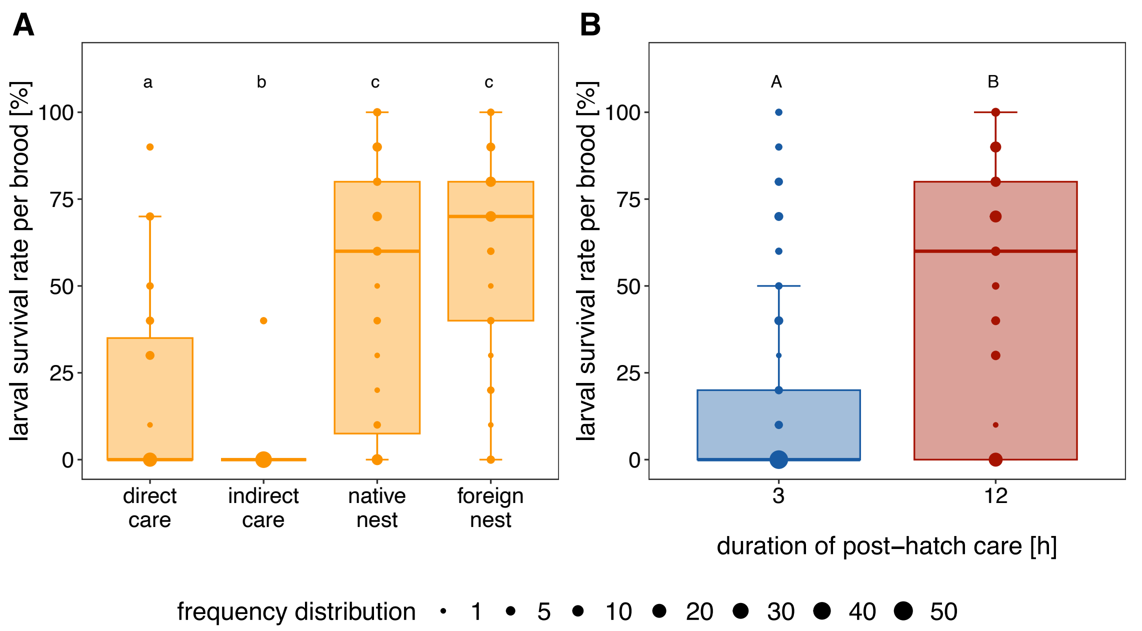

Figure 3.4
Larval survival ratio at dispersal in relation to (A) type of care and (B) duration of care. Larvae received either 3 h or 12 h of post-hatching care and one of four types of parental care: ‘direct care’ (3 h: n = 18; 12 h: n = 17), ‘indirect care’ (3 h: n = 18; 12 h: n = 17), ‘native nest environment’ (3 h: n = 16; 12 h: n = 18), or ‘foreign nest environment’ (3 h: n = 16; 12 h: n = 17). Boxplots show median and the interquartile range and the data point size equals the number of broods with the same survival. Different letters indicate significant differences among the four levels of (A) types of care or between the two levels of (B) duration of post-hatch care.

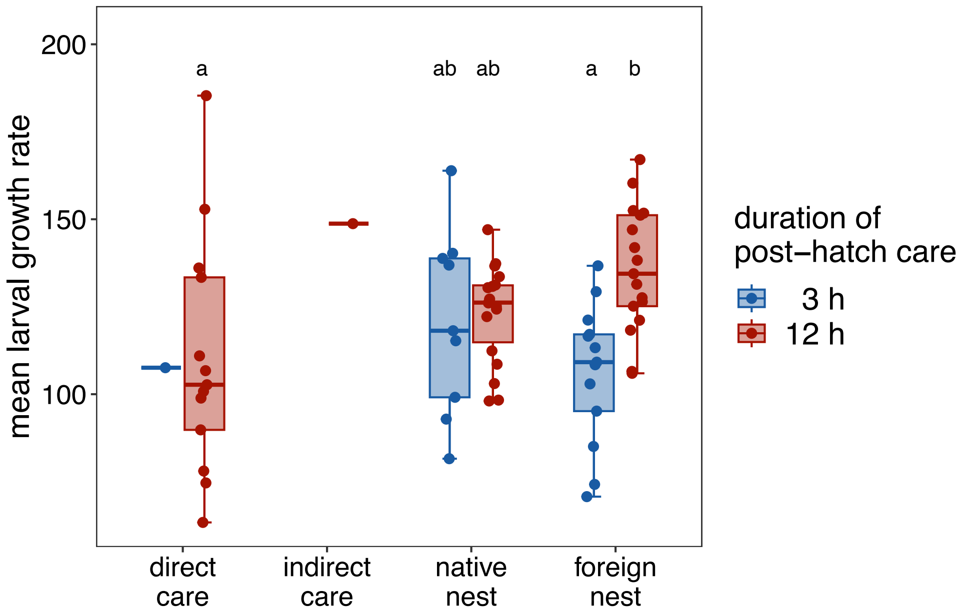

Figure 3.5
Mean growth rate of the surviving larvae at dispersal in relation to type of care and duration of care. Larvae received either 3 h or 12 h of post-hatching care and one of four types of parental care: 'direct care' (3 h: n = 1; 12 h: n = 13), 'indirect care' (3 h: n = no surviving broods; 12 h: n = 1), 'native nest environment' (3 h: n = 9; 12 h: n = 18), or 'foreign nest environment' (3 h: n = 13; 12 h: n = 17). Boxplots show median and the interquartile range and the data point size equals the number of broods with the same survival. Different letters indicate significant between-group differences. Groups with one single value or less were excluded from these analyses.

Larval performance at dispersal

At dispersal, larval survival ratio was influenced by the duration of post-hatching care, by the type of care, but not by their interaction (**Table 3.2, Fig. 3.4A**). Larval survival ratio differed among the type of care larvae received (**Fig. 3.4A**) and again larvae survived better with an increase in the duration of parental care (**Fig. 3.4B**).

Similar to larval performance at 24 h, larvae survived equally well irrespective of whether they were relocated to a 'foreign nest environment' or remained in their 'native nest environment', but both groups survived better than larvae that received 'direct care' followed by larvae receiving only 'indirect care' (**Fig. 3.4A**). Note that larvae receiving only 'indirect care' did not survive at all, regardless of the duration of care, neither did larvae that received only 3 h of 'direct care' (**Fig. 3.4A**).

In contrast to larval survival, larval growth rate was only influenced by the interaction of the duration and type of post-hatching care (**Table 3.2**). Larvae that remained in their 'native nest environment' grew equally fast irrespective of whether they received 3 or 12 h of post-hatching care and did not differ from any of the other treatments (**Fig. 3.5**). However, larvae that were relocated to a 'foreign nest environment' and that received 12 h of post-hatching care grew faster than their 3 h counterpart and faster than larvae receiving 12 h of 'direct care'. There was no difference between larvae receiving 3 h of care and a 'foreign nest environment' and larvae receiving 12 h of 'direct care' (**Fig. 3.5**). We had to exclude three larval treatment groups ('3 h of direct care', '3 h of indirect care' and '12 h of indirect care', see **Fig. 3.4A**) from the larval growth rate analyses because in each of these groups only one brood had surviving larvae.

Discussion

This study aimed to provide new insights into the importance of duration and type of care for offspring growth and survival in a species with obligate parental care. Our results show that both larval survival and growth rate increased with the duration of post-hatch care. Larval survival was highest when larvae were exposed to at least 12 hours of direct care or a combination of direct and indirect care, whereas shorter periods of care or only indirect care, i.e., parental modification of the nest, resulted in high larval mortality. Direct care had a much stronger effect on promoting larval growth and survival than indirect care. Thus, we were able to show that parental regurgitation is important for offspring survival, but post-hatching manipulation of the carrion resource is also essential to ensure optimal larval performance.

The duration of parental care varies widely across species and can range from just a few hours to several years (Clutton-Brock 1991; Royle, Smiseth & Kölliker 2012). However, how much of the total care time is crucial for offspring survival is often unknown. Although *N. orbicollis* parents provide around 120 hours of post-hatching care and feed their offspring with regurgitated carrion for at least 48 hours, an earlier study has shown that already 3 hours are enough for some larvae to survive in the absence of competitors or predators (Capodeanu-Nägler *et al.* 2018). Our study corroborates this earlier finding, as we found that some larval survival can be achieved with as little as 3 hours of exposure to post-hatching care. In addition, we show that both larval survival as well as larval growth rates increase with care duration in the initial 12 hours after hatching. Such an improvement of larval performance within the first 12 hours was also reported by the study of Capodeanu-Nägler *et al.* (2018). Even in *N. vespilloides*, in which care is facultative and offspring can partially survive without the help of the parents, larval performance increased in the first 12 hours of care (Eggert, Reinking & Müller 1998). After this period of post-hatching care, however, the benefit of care declined considerably in both species. Although parental removal experiments have been performed in a range of species across different taxa to establish the adaptive significance of parental care, there are much fewer studies which explored the role of the duration of care, and those studies are often done in vertebrates. Here, the length of care that is essential for offspring survival certainly varies greatly between species and depends on the development mode and the harshness of their environment. In the glassfrog *Ikakogi tayrona,* for example*,* females care for their eggs for several days. A female removal experiment revealed that the first day of care substantially reduced the risk of embryo mortality due to dehydration (Valencia & Delia 2016). However, after this period the benefit of care to embryo survival declined substantially. That the benefit of care decreases with embryonic development was also observed in four other glassfrog species (Delia, Bravo-Valencia & Warkentin 2020). There are also studies which show that even prolonged periods of care can have positive effects on offspring survival. In the red deer, for example, orphaning was associated with a reduced survival even after 12 month of care (Andres *et al.* 2013) and a study of a neotropical passerine, the western slaty-antshrike (*Thamnophilus atrinucha*), found that post-fledging parental care correlated with higher offspring survival (Tarwater & Brawn 2010). A study in leopards (*Panthera pardus*), on the other hand, found no correlation between the duration of maternal care and post-independence survival or reproductive success of offspring (Balme *et al.* 2017).

The study by Capodeanu-Nägler *et al.* (2016) found no effect of pre-hatching care on offspring survival in *N. orbicollis* but found that offspring survival was critically dependent on post-hatching care. However, since already 3 hours of post-hatching care dramatically increased offspring survival (Capodeanu-Nägler et al. 2018), it remained unclear whether this marked increase in survival was primarily due to direct parental feeding or whether the parental modification of the feeding cavity during the post-hatching phase - and thus indirect care - played a more pivotal or at least an additional role. Our results clearly show that when considering only the first three hours of care, both direct and indirect care are needed for larvae to survive. However, our results also demonstrates that direct care is more important than indirect care. When provided with a carrion resource that had received 12 hours of indirect care, offspring were not able to survive, but with the same amount of direct care, some larvae were able to survive even when given an unprepared carrion resource.

Supplying proteins and yolk lipids to eggs, or depositing eggs on or into nutritional resources, are much more common parental strategies across the animal kingdom than direct offspring provisioning. While direct provisioning is ubiquitous in mammals and nearly so in birds, it only occurs in about 1 % of insect species (Costa 2006; Balshine 2012; Royle, Russell & Wilson 2014). Direct parental feeding is both time consuming and energetically demanding and so its evolution is favored only under certain conditions, for example when parents are more efficient in offspring provisioning than offspring in self-feeding (Gardner & Smiseth 2011). It is also favored when safe nest sides that protect offspring from predators are spatially separated from food sources, such as nests high up in trees. For burying beetles, however, the nest itself serves as the food source, negating the relevance of the latter factor in driving the evolution of parental feeding. However, it is quite likely that parents are much more efficient at predigesting food than their offspring. We must also consider that the oral fluids transferred from parents to offspring have been shown to contain not only predigested food but also other materials, such as enzymes, antimicrobial agents, and microbes (Hoback *et al.* 2004; Degenkolb, Düring & Vilcinskas 2011; Shukla, Vogel*, et al.* 2018; Körner, Steiger & Shukla 2023). They may even contain hormones or growth factors (see e.g., LeBoeuf *et al.* 2016; Hakala *et al.* 2023). One or more of these transferred components may be critical for larval growth. In fact, (Capodeanu-Nägler *et al.* 2018) demonstrated that larval survival increased when oral secretions were supplemented to a diet of liquefied carrion.

Although direct care had a larger effect on larval performance, indirect care also had a positive effect on offspring growth and survival. During the post-hatching phase, parents continue to coat the outer surface of the carrion resource with antimicrobial secretions. However, we do not believe that this is the key factor explaining our result. As mentioned before, Capodeanu-Nägler *et al.* (2016) found no effect of pre-hatching care on larval performance and there is currently no evidence that the treatment of the outer surface is much different in the pre- and in the post-hatching phases. However, what is likely to be an important factor is the parental manipulation of the feeding cavity in which the larvae reside. Parents can often be seen sticking their heads into the cavity or crawling into it with their whole body. This is mainly done to feed from the flesh. However, at least in *N. vespilloides* it has been shown that the feeding cavity is characterized by a biofilm-like matrix containing *Yarrowia* and other beetle-derived microbes that promote larval development (Shukla, Plata*, et al.* 2018). Daily removal of the matrix had a negative effect on larval growth even in the presence of direct care. Consequently, it has been suggested that the feeding cavity serves as an extraintestinal site for nutrient processing mediated by beetle-transferred microbial symbionts (Shukla, Vogel*, et al.* 2018). The same might be true for the feeding cavity of *N. orbicollis*, at least the adults are also known to harbor *Yarrowia* (Kaltenpoth & Steiger 2014).

Based on our experiment, we cannot rule out that the beneficial effects we observed were not exclusively due to indirect parental care, but also to the previous larvae that were present in the feeding cavity. They might have contributed to the manipulation of the cavity, e.g., by depositing oral and anal fluids characterized by digestive enzymatic and antimicrobial activities (Arce, Smiseth

& Rozen 2013; Reavey, Beare & Cotter 2014). Larval-derived benefits have been observed in other carrion insects and have been shown to play a role in *Nicrophorus* – at least under certain conditions (Schrader, Jarrett & Kilner 2015a; Magneville *et al.* 2018; Rebar *et al.* 2020; Prang *et al.* 2022).

Additionally, our results indicate that larvae derive some small benefit from being relocated to another modified nest. While the growth rate at 24 hours and up until dispersal showed differences between larvae receiving direct care and control larvae that obtained a foreign modified nest, there was no such difference when compared to larvae that remained on their original carrion resource. This outcome might be attributed to the altered spatial access to the feeding cavity following relocation. Previously disadvantaged larvae might have found better feeding positions within the feeding cavity, potentially enhancing overall food intake. Another explanation lies within the microbiome. In addition to protecting offspring from environmental hazards, nest construction has been demonstrated to play a pivotal role in shaping the offspring's microbiome in various animal species (Campos-Cerda & Bohannan 2020). Similarly, in burying beetles, a core microbiota is transferred to the offspring (Vogel *et al.* 2017; Körner, Steiger & Shukla 2023). This transfer occurs through direct trophallaxis, as well as from the parents' oral and anal secretions applied to the carcass. As a result, exposure to the microbiome in a different feeding cavity may enrich the larval microbiome. A more diverse microbiome has been shown to be beneficial in other insect species (Segers, Kaltenpoth & Foitzik 2019; Lange *et al.* 2023).

In conclusion, our study highlights the crucial role of food provisioning in early larval development and survival. Our results indicate that, direct parental feeding significantly influences larval performance. However, if parents leave before larvae attain nutritional independence, the importance of indirect care, such as nest modification and the alteration of the feeding cavity, becomes more apparent. Whilst our results confirm the importance of direct food provisioning for larval survival, we emphasize the significant supportive role of post-hatching carcass modification in larval growth. In addition, it is essential to highlight that due to parent-offspring coadaptation, the importance of direct and indirect care can undergo significant shifts over evolutionary time (Royle, Alonzo & Moore 2016). For instance, while in *N. orbicollis*, survival is critically tied to post-hatching care, this is not observed across all burying beetle species (Trumbo 1992; Capodeanu-Nägler *et al.* 2016). This pattern suggests that the distinct advantage derived from parental feeding in *N. orbicollis* may stem from an evolved dependency, rather than representing the benefit in the ancestral state. The greater reliance on feeding than on indirect care may be the result of sibling competition for food, which has been predicted to reinforce the evolution of parental provisioning and in turn to increase offspring dependency (Gardner & Smiseth 2011;

Royle, Alonzo & Moore 2016). However, more work is needed to better understand the complex co-evolutionary dynamics between parental and offspring traits and how the benefits of different care components change once care originated. In birds and mammals, for example, indirect care, i.e., the construction of a protective nest, has strong consequences for offspring development mode which can reinforce the evolution of direct care and thus increase the benefits of direct care. In burying beetles, similar patterns might have emerged in which both direct and indirect care collectively influence larval success. The dynamic interplay between these care strategies highlights the multifaceted and complex nature of parental care and its evolutionary implications.

References

Andres, D., Clutton-Brock, T.H., Kruuk, L.E.B., Pemberton, J.M., Stopher, K.V. & Ruckstuhl, K.E. (2013) Sex differences in the consequences of maternal loss in a long-lived mammal, the red deer (*Cervus elaphus*). *Behavioral Ecology and Sociobiology,* 67, 1249-1258. https://doi.org/10.1007/s00265-013-1552-3

Arce, A.N., Smiseth, P.T. & Rozen, D.E. (2013) Antimicrobial secretions and social immunity in larval burying beetles, *Nicrophorus vespilloides*. *Animal Behaviour,* 86, 741-745. https://doi.org/10.1016/j.anbehav.2013.07.008

Balme, G.A., Robinson, H.S., Pitman, R.T. & Hunter, L.T.B. (2017) Flexibility in the duration of parental care: Female leopards prioritise cub survival over reproductive output. *Journal of Animal Ecology,* 86, 1224-1234. https://doi.org/10.1111/1365-2656.12713

Balshine, S. (2012) Patterns of parental care in vertebrates. *The evolution of parental care* (eds N.J. Royle, P.T. Smiseth & M. Kölliker), pp. 62-80. Oxford Universtiy Press, Oxford, U.K., https://doi.org/10.1093/acprof:oso/9780199692576.003.0004

Baur, B. (1994) Parental care in terrestrial gastropods. *Experientia,* 50, 5-14. https://doi.org/10.1007/BF01992042

Caccavo, D., Pellegrino, N.M., Altamura, M., Rigon, A., Amati, L., Amoroso, A. & Jirillo, E. (2002) Antimicrobial and immunoregulatory functions of lactoferrin and its potential therapeutic application. *Journal of Endotoxin Research,* 8, 403-417.

Campos-Cerda, F. & Bohannan, B.J.M. (2020) The nidobiome: A framework for understanding microbiome assembly in neonates. *Trends in Ecology & Evolution,* 35, 573-582. https://doi.org/10.1016/j.tree.2020.03.007

Capodeanu-Nägler, A., Keppner, E.M., Vogel, H., Ayasse, M., Eggert, A.-K., Sakaluk, S.K. & Steiger, S. (2016) From facultative to obligatory parental care: Interspecific variation in offspring dependency on post-hatching care in burying beetles. *Scientific Reports,* 6, 29323. https://doi.org/10.1038/srep29323

Capodeanu-Nägler, A., Prang, M.A., Trumbo, S.T., Vogel, H., Eggert, A.-K., Sakaluk, S.K. & Steiger, S. (2018) Offspring dependence on parental care and the role of parental transfer of oral fluids in burying beetles. *Frontiers in Zoology,* 15, 1-12. https://doi.org/10.1186/s12983-018-0278-5

Clutton-Brock, T.H. (1991) *The evolution of parental care*. Princeton University Press, Princeton, New Jersey, USA, https://doi.org/10.1046/j.1420-9101.1992.5040719.x

Costa, J.T. (2006) *The other insect societies*. Harvard University Press, Cambridge, Massachusetts, USA,

Cotter, S.C. & Kilner, R.M. (2010a) Personal immunity versus social immunity. *Behavioral Ecology,* 21, 663-668. https://doi.org/10.1093/beheco/arq070

Degenkolb, T., Düring, R.-A. & 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. https://doi.org/10.1007/s10886-011-9978-4

Delia, J., Bravo-Valencia, L. & Warkentin, K.M. (2020) The evolution of extended parental care in glassfrogs: Do egg-clutch phenotypes mediate coevolution between the sexes? *Ecological Monographs,* 90, e01411. https://doi.org/10.1002/ecm.1411

Eggert, A.-K. & Müller, J.K. (1997) Biparental care and social evolution in burying beetles: Lessons from the larder. *The evolution of social behavior in insects and arachnids* (eds J.C. Choe & B.J. Crespi), pp. 216-236. Cambridge, Massachusetts, U.S.A, https://doi.org/10.1017/CBO9780511721953.011

Eggert, A.-K., Reinking, M. & Müller, J.K. (1998) Parental care improves offspring survival and growth in burying beetles. *Animal Behaviour,* 55, 97-107. https://doi.org/10.1006/anbe.1997.0588

Eraud, C., Dorie, A., Jacquet, A. & Faivre, B. (2008) The crop milk: A potential new route for carotenoid-mediated parental effects. *J Avian Biol,* 39, 247-251. https://doi.org/10.1111/j.0908-8857.2008.04053.x

Gardner, A. & Smiseth, P.T. (2011) Evolution of parental care driven by mutual reinforcement of parental food provisioning and sibling competition. *Proceedings of the Royal Society B: Biological Sciences,* 278, 196-203. https://doi.org/10.1098/rspb.2010.1171

Goldman, A.S. (1993) The immune system of human milk: Antimicrobial, antiinflammatory and immunomodulating properties. *The Pediatric Infectious Disease Journal,* 12, 664-672.

Grubbauer, P. & Hoi, H. (1996) Female penduline tits (*Remiz pendulinus*) choosing high quality nests benefit by decreased incubation effort and increased hatching success. *Écoscience,* 3, 274-279. https://doi.org/10.1080/11956860.1996.11682342

Hakala, S.M., Fujioka, H., Gapp, K., De Gasperin, O., Genzoni, E., Kilner, R.M., Koene, J.M., König, B., Linksvayer, T.A., Meurville, M.-P., Negroni, M.A., Palejowski, H., Wigby, S. & LeBoeuf, A.C. (2023) Socially transferred materials: Why and how to study them. *Trends in Ecology & Evolution,* 38, 446-458. https://doi.org/10.1016/j.tree.2022.11.010

Hall, C.L., Wadsworth, N.K., Howard, D.R., Jennings, E.M., Farrell, L.D., Magnuson, T.S. & Smith, R.J. (2011) Inhibition of microorganisms on a carrion breeding resource: The antimicrobial peptide activity of burying beetle (Coleoptera: Silphidae) oral and anal secretions. *Environmental Entomology,* 40, 669-678. https://doi.org/10.1603/EN10137

Hoback, W.W., Bishop, A.A., Kroemer, J., Scalzitti, J. & Shaffer, J.J. (2004) Differences among antimicrobial properties of carrion beetle secretions reflect phylogeny and ecology. *Journal of Chemical Ecology,* 30, 719-729. https://doi.org/10.1023/b:joec.0000028427.53141.41

Kaltenpoth, M., Göttler, W., Herzner, G. & Strohm, E. (2005) Symbiotic bacteria protect wasp larvae from fungal infestation. *Current Biology,* 15, 475-479. https://doi.org/10.1016/j.cub.2004.12.084

Kaltenpoth, M. & Steiger, S. (2014) Unearthing carrion beetles' microbiome: Characterization of bacterial and fungal hindgut communities across the Silphidae. *Molecular Ecology,* 23, 1251-1267. https://doi.org/10.1111/mec.12469

Kleiman, D.G. & Malcolm, J.R. (1981) The evolution of male parental investment in mammals. *Parental care in mammals*, pp. 347-387. Springer,

Körner, M., Steiger, S. & 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. https://doi.org/10.3389/fevo.2023.1252876

Kramer, J. & Meunier, J. (2019) The other facets of family life and their role in the evolution of animal sociality. *Biological Reviews,* 94, 199-215. https://doi.org/10.1111/brv.12443

Lange, C., Boyer, S., Bezemer, T.M., Lefort, M.-C., Dhami, M.K., Biggs, E., Groenteman, R., Fowler, S.V., Paynter, Q., Verdecia Mogena, A.M. & Kaltenpoth, M. (2023) Impact of intraspecific variation in insect microbiomes on host phenotype and evolution. *The ISME Journal.* https://doi.org/10.1038/s41396-023-01500-2

LeBoeuf, A.C., Waridel, P., Brent, C.S., Gonçalves, A.N., Menin, L., Ortiz, D., Riba-Grognuz, O., Koto, A., Soares, Z.G., Privman, E., Miska, E.A., Benton, R. & Keller, L. (2016) Oral transfer of chemical cues, growth proteins and hormones in social insects. *Elife,* 5. https://doi.org/10.7554/eLife.20375

Magneville, C., Ratz, T., Richardson, J. & Smiseth, P.T. (2018) No evidence of sibling cooperation in the absence of parental care in *Nicrophorus vespilloides*. *Evolution,* 72, 2803-2809. https://doi.org/10.1111/evo.13622

Mennerat, A., Mirleau, P., Blondel, J., Perret, P., Lambrechts, M.M. & Heeb, P. (2009) Aromatic plants in nests of the blue tit *Cyanistes caeruleus* protect chicks from bacteria. *Oecologia,* 161, 849-855. https://doi.org/10.1007/s00442-009-1418-6

Meunier, J., Körner, M. & Kramer, J. (2022) Parental care. *Reproductive Strategies in Insects*, pp. 337-357. https://doi.org/10.1201/9781003043195-17

Milani, C., Duranti, S., Bottacini, F., Casey, E., Turroni, F., Mahony, J., Belzer, C., Delgado Palacio, S., Arboleya Montes, S., Mancabelli, L., Lugli, G.A., Rodriguez, J.M., Bode, L., de Vos, W., Gueimonde, M., Margolles, A., van Sinderen, D. & Ventura, M. (2017) The first microbial colonizers of the human gut: Composition, activities, and health implications of the infant gut microbiota. *Microbiology and Molecular Biology Reviews,* 81, e00036-00017. https://doi.org/10.1128/MMBR.00036-17

Miller, C.J., Bates, S.T., Gielda, L.M. & Creighton, J.C. (2019) Examining transmission of gut bacteria to preserved carcass via anal secretions in *Nicrophorus defodiens*. *PLoS One,* 14, e0225711. https://doi.org/10.1371/journal.pone.0225711

Milne, L.J. & Milne, M. (1976) The social behavior of burying beetles. *Scientific American,* 235, 84-89. https://doi.org/10.1038/scientificamerican0876-84

Müller, J.K. & Eggert, A.-K. (1990) Time-dependent shifts between infanticidal and parental behavior in female burying beetles: A mechanism of indirect mother-offspring recognition. *Behavioral Ecology and Sociobiology,* 27, 11-16. https://doi.org/10.1007/BF00183307

Prang, M.A., Zywucki, L., Körner, M. & Steiger, S. (2022) Differences in sibling cooperation in presence and absence of parental care in a genus with interspecific variation in offspring dependence. *Evolution,* 76, 320-331. https://doi.org/10.1111/evo.14414

Pukowski, E. (1933) Ökologische Untersuchungen an *Necrophorus* F. *Zeitschrift für Morphologie und Ökologie der Tiere,* 27, 518-586. https://doi.org/10.1007/BF00403155

Pukowski, E. (1934a) Die Brutpflege des Totengräbers. *Entomologische Blätter,* 30, 109-113.

Reavey, C.E., Beare, L. & Cotter, S.C. (2014) Parental care influences social immunity in burying beetle larvae. *Ecological Entomology,* 39, 395-398. https://doi.org/10.1111/een.12099

Rebar, D., Bailey, N.W., Jarrett, B.J.M. & Kilner, R.M. (2020) An evolutionary switch from sibling rivalry to sibling cooperation, caused by a sustained loss of parental care. *Proceedings of the National Academy of Sciences,* 117, 2544-2550. https://doi.org/10.1073/pnas.1911677117

Royle, N.J., Alonzo, S.H. & Moore, A.J. (2016) Co-evolution, conflict and complexity: What have we learned about the evolution of parental care behaviours? *Current opinion in behavioral sciences,* 12, 30-36. https://doi.org/10.1016/j.cobeha.2016.08.004

Royle, N.J., Russell, A.F. & Wilson, A.J. (2014) The evolution of flexible parenting. *Science,* 345, 776-781. https://doi.org/10.1126/science.1253294

Royle, N.J., Smiseth, P.T. & Kölliker, M. (2012) *The evolution of parental care*. Oxford University Press, Oxford, U.K., https://doi.org/10.1093/acprof:oso/9780199692576.001.0001

Ruiz-Castellano, C., Tomás, G., Ruiz-Rodríguez, M., Martín-Gálvez, D. & Soler, J.J. (2016) Nest material shapes eggs bacterial environment. *PLoS One,* 11, e0148894. https://doi.org/10.1371/journal.pone.0148894

Schrader, M., Jarrett, B.J. & Kilner, R.M. (2015a) Parental care masks a density-dependent shift from cooperation to competition among burying beetle larvae. *Evolution,* 69, 1077-1084. https://doi.org/10.1111/evo.12615

Segers, F.H.I.D., Kaltenpoth, M. & Foitzik, S. (2019) Abdominal microbial communities in ants depend on colony membership rather than caste and are linked to colony productivity. *Ecology and Evolution,* 9, 13450-13467. https://doi.org/10.1002/ece3.5801

Shukla, S.P., Plata, C., Reichelt, M., Steiger, S., Heckel, D.G., Kaltenpoth, M., Vilcinskas, A. & Vogel, H. (2018) Microbiome-assisted carrion preservation aids larval development in a burying beetle. *Proceedings of the National Academy of Sciences,* 115, 11274-11279. https://doi.org/10.1073/pnas.1812808115

Shukla, S.P., Vogel, H., Heckel, D.G., Vilcinskas, A. & Kaltenpoth, M. (2018) Burying beetles regulate the microbiome of carcasses and use it to transmit a core microbiota to their offspring. *Molecular Ecology,* 27, 1980-1991. https://doi.org/10.1111/mec.14269

Smiseth, P.T., Kölliker, M. & Royle, N.J. (2012) What is parental care? *The evolution of parental care* (eds N.J. Royle, P.T. Smiseth & M. Kölliker), pp. 01-17. Oxford University Press, Oxford, U.K., https://doi.org/10.1093/acprof:oso/9780199692576.003.0001

Socias-Martínez, L. & Kappeler, P.M. (2019) Catalyzing transitions to sociality: Ecology builds on parental care. *Frontiers in Ecology and Evolution,* 7. https://doi.org/10.3389/fevo.2019.00160

Tarwater, C.E. & Brawn, J.D. (2010) The post-fledging period in a tropical bird: Patterns of parental care and survival. *J Avian Biol,* 41, 479-487. https://doi.org/10.1111/j.1600-048X.2010.05006.x

Trumbo, S.T. (1992) Monogamy to communal breeding: Exploitation of a broad resource base by burying beetles (*Nicrophorus*). *Ecological Entomology,* 17, 289-298. https://doi.org/10.1111/j.1365-2311.1992.tb01060.x

Trumbo, S.T. (2012) Patterns of parental care in invertebrates. *The evolution of parental care* (eds N.J. Royle, P.T. Smiseth & M. Kölliker), pp. 81-100. Oxford University Press, Oxford, U.K., https://doi.org/10.1093/acprof:oso/9780199692576.003.0005

Valencia, L.B. & Delia, J. (2016) Maternal care in a glassfrog: Care function and commitment to offspring in *Ikakogi tayrona*. *Behavioral Ecology and Sociobiology,* 70, 41-48. https://doi.org/10.1007/s00265-015-2022-x

Vogel, H., Shukla, S.P., Engl, T., Weiss, B., Fischer, R., Steiger, S., Heckel, D.G., Kaltenpoth, M. & Vilcinskas, A. (2017) The digestive and defensive basis of carcass utilization by the burying beetle and its microbiota. *Nature Communications,* 8, 1-15. https://doi.org/10.1038/ncomms15186

Wang, Y. & Rozen, D.E. (2018) Gut microbiota in the burying beetle, *Nicrophorus vespilloides*, provide colonization resistance against larval bacterial pathogens. *Ecology and Evolution,* 8, 1646-1654. https://doi.org/10.1002/ece3.3589

Winkler, D.W. & Sheldon, F., H. (1993) Evolution of nest construction in swallows (Hirundinidae): a molecular phylogenetic perspective. *Proceedings of the National Academy of Sciences,* 90, 5705-5707. doi:10.1073/pnas.90.12.5705

Publication 4: Differences in sibling cooperation in presence and absence of parental care in a genus with interspecific variation in offspring dependence

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M.A.P., L.Z., and S.S. designed the experiment. M.A.P. and L.Z. conducted the experiment. All authors analyzed the data. M.A.P. wrote the first draft of the manuscript. All authors revised it until completion.

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Abstract

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.

Introduction

The taxonomically widespread phenomenon of family life, i.e., the association of offspring with their parents after birth or hatching (Kramer & Meunier 2019), is considered an important first step in the evolution of sociality (Clutton-Brock 1991; Royle, Smiseth & Kölliker 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, Alonzo & Bonsall 2012; Wong, Meunier & Kölliker 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 & 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 & Meunier 2019).

Indeed, recent years have seen increasing attention towards 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, Griffin & Gardner 2007b). Sibling cooperation is promoted by kinship (Hamilton 1964a; Hamilton 1964b; West, Griffin & Gardner 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, Sakaluk & Thompson 2013), coordinated begging in banded mongooses (*Mungo mungo*; Bell 2007), and social immunity behaviors in eusocial insects (Cremer, Armitage & Schmid-Hempel 2007; Stow *et al.* 2007; Hamilton, Lejeune & Rosengaus 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 & Rayor 2013), black lace-weavers *(Amaurobius ferox*; Kim, Krafft & Choe 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, Thesing & Meunier 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 towards prolonged forms of family life (Kramer & 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 & Müller 1997). Prehatching care is mainly comprised 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 & Kilner 2010a; 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, Reinking & Müller 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; Pukowski 1934a; Milne & 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, Smiseth & Rozen 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, Jarrett & Kilner 2015a). 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 non-prepared carcass with a standardized feeding cavity and (2) a parentally prepared carcass. Even though non-prepared 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 pre-digestion (Wang & 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. Since previous studies detected cooperative behavior only in the absence of posthatching parental care (Schrader, Jarrett & Kilner 2015a), 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 hours 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. In order to be able to compare our findings with previous efforts to investigate sibling cooperation (Schrader, Jarrett & Kilner 2015a; 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 intermediary 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. Since 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; Capodeanu-Nägler *et al.* 2018). Secondly and more importantly, if cooperation depends on the degree of offspring reliance on parental care (Kramer & 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. Thirdly, 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), since harsh environments have been shown to promote mutually beneficial interactions (Falk *et al.* 2014; Schrader, Jarrett & Kilner 2015a; Kramer & 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); *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 x 10 x 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 2 and 3 generations, *N. orbicollis* were reared for 9 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 non-prepared 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, non-sibling virgin males and females were paired haphazardly in small boxes (10 x 10 x 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. 24 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 hours) 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, e.g., in the house wren (*Troglodytes aedon*; Bowers, Sakaluk & Thompson 2013), the European earwig (*Forficula auricularia*; Kramer, Thesing & Meunier 2015) and also burying beetles (Smiseth, Darwell & Moore 2003; Rebar *et al.* 2020). Larval mass was determined to 0.01 mg using a precision scale (Kern ABJ120-4NM, Kern und Sohn GmbH, Balingen, Germany). We placed 1, 2, 3, 5, 10, or 15 larvae on one of two carcass types: either a non-prepared or a parentally prepared carcass. To obtain parentally prepared carcasses, we separated the above-mentioned 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, non-prepared 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). Since 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 supplemental Fig. S1 for the survival rate per brood until dispersal for all three species), we measured larval performance at hatching and 24 hours 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. In order to be able to compare our findings with previous efforts to investigate sibling cooperation (Schrader, Jarrett & Kilner 2015a; Magneville *et al.* 2018), we additionally measured larval mass and survival in *N. vespilloides* at the time of dispersal, i.e., 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 $(lm_{24} - lm_0)/ lm_0$, 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 quasi-binomial 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 (non-prepared 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 of the ‘car’ package. Since we found significant two-way interactions between carcass type and species and carcass type and brood size, we split the data set 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. (2015a), 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 quasi-binomial distribution and a logit link function. As we were particularly interested in whether larvae performed differently on each carcass type, we split the data set 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

Table 4.1

Results of generalized linear models (fitted with a Gaussian error distribution for growth rate and quasi-binomial 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 hours 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	500			376.17	696		

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 4.1**) and survival rate per brood (**Table 4.1**). 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. 4.1A** and **4.2A**). The larvae of the intermediary dependent species *N. vespilloides* showed a corresponding intermediary growth and survival rate.

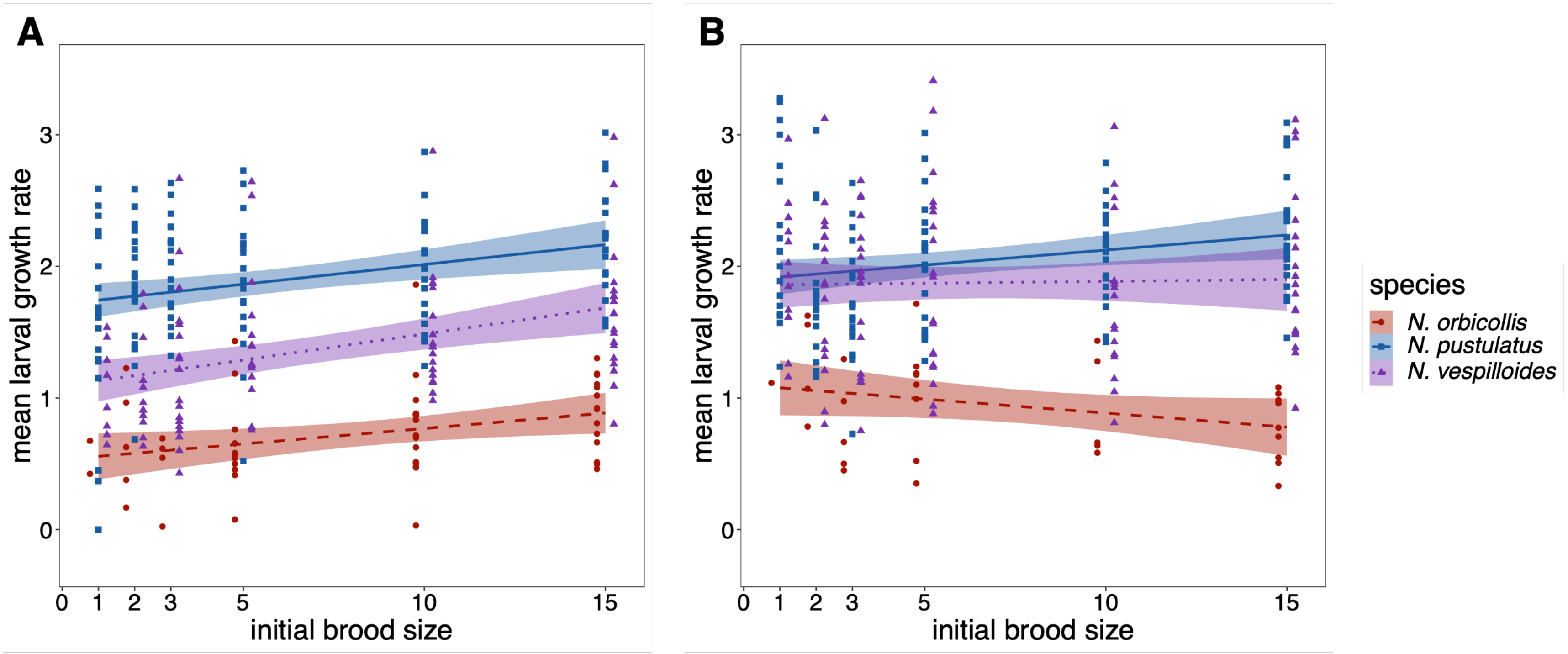

Figure 4.1

The relationship between initial brood size and mean larval growth rate per brood in the first 24 hours after hatching on (A) non-prepared 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. (© Figure adapted from (Prang *et al.* 2022))

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 4.1**). Whereas on non-prepared carcasses, larval growth and survival rate increased with an increase of brood size, larval growth and survival were not affected by brood size on prepared carcasses (**Fig. 4.1** and **Fig. 4.2**). 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 4.1**). 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 non-prepared carcasses (**Fig. 4.1A; Table 4.2**). 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. 4.1B; Table 4.2**). Consequently, the detected interaction effect between brood size and carcass type on larval growth rate (**Table 4.1**) 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. 4.2B; Table 4.3**). On non-prepared carcasses, we detected a positive effect of brood size on larval survival rate in *N. orbicollis* and *N. vespilloides* (**Fig. 4.2A; Table 4.3**). In *N. pustulatus*, no such effect could be revealed (**Fig. 4.2A; Table 4.3**). Hence, also regarding the survival rate, the detected interaction effect between brood size and carcass type (**Table 4.1**) was driven by *N. orbicollis* and *N. vespilloides* larvae. However, we emphasize that in *N. pustulatus*, it was not possible to detect any effect because nearly all larvae survived under both conditions (**Fig. 4.2**).

Table 4.2

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 hours after hatching in the three 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, while 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 4.3

Results of generalized linear models (fitted with a quasi-binomial 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 hours 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, while 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 4.4
Results of generalized linear models (fitted with a Gaussian error distribution for mean larval mass and quasi-binomial 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.

	Growth rate				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**
Carcass type * Brood size	774	1	1.76	0.19	2.43	1	5.15	**0.024**
Residuals	58823	134			106.16	225		

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 4.4**). However, separated GLMs for each carcass type revealed that this effect was driven by larvae on non-prepared carcasses since *N. vespilloides* larvae only gained more weight with an increase of brood size on non-prepared carcasses (**Fig. 4.3A; Table 4.5**).

Similarly, the survival rate until dispersal was also affected by brood size. However, here the effect depended on carcass type (**Fig. 4.3B; Table 4.4**): separated GLMs for each carcass type revealed that only on non-prepared carcasses larval survival increased with an increase in brood size (**Table 4.5**).

Finally, we examined the effect of the number of dispersing offspring on larval mass at dispersal (**Fig. S4.2**). Separated GLMs for each carcass type revealed that the number of dispersed offspring affected larval mass positively on both carcass types (**Table S4.1**).

Table 4.5

Results of generalized linear models (fitted with a Gaussian error distribution for mean larval mass and quasi-binomial 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, while 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	< 0.001
	Brood size	0.14	0.032	113	4.48	**< 0.001**	0.044	0.028	112	1.58	0.78
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	0.81

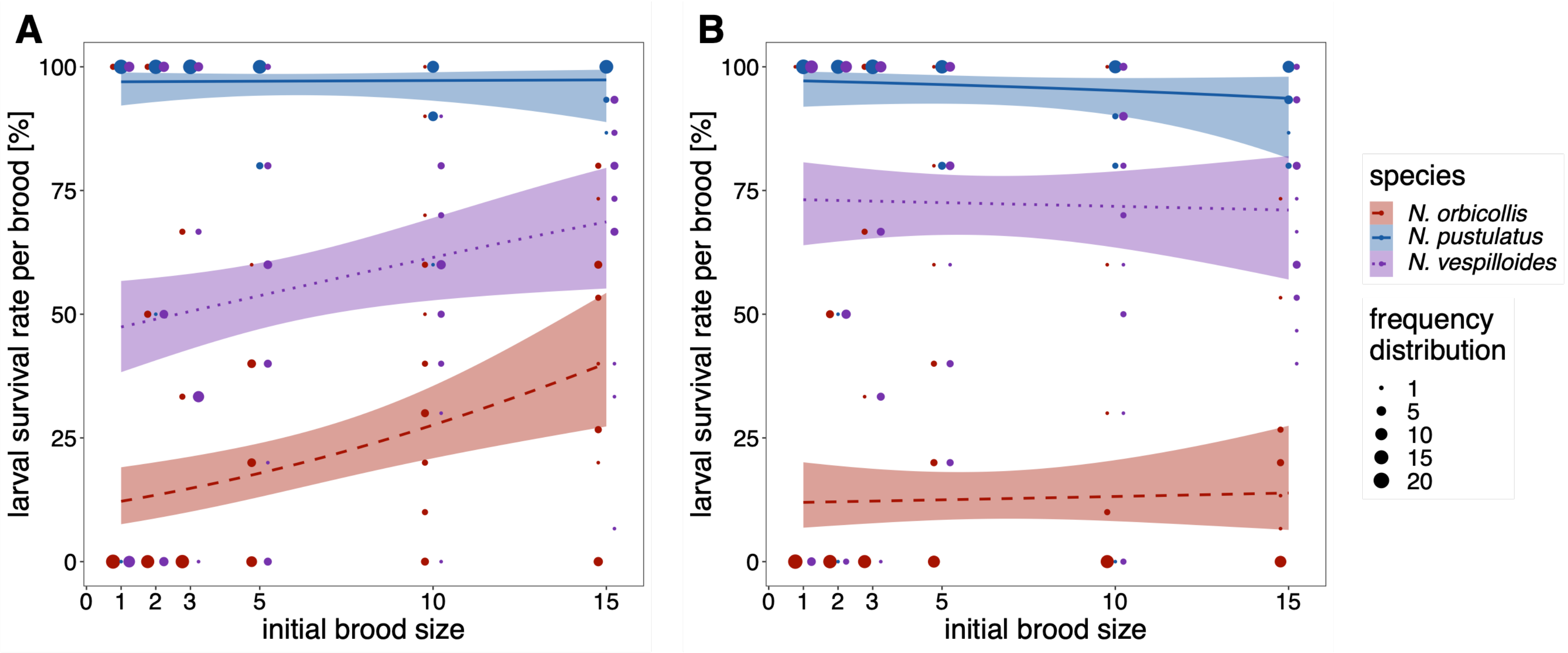

Figure 4.2
The relationship between initial brood size and larval survival rate per brood in the first 24 hours after hatching on (A) non-prepared carcasses and (B) parentally prepared carcasses in the three burying beetle species *N. orbicollis* (n = 240), *N. pustulatus* (n =240), and *N. vespilloides* (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. (©Figure adapted from (Prang *et al.* 2022))

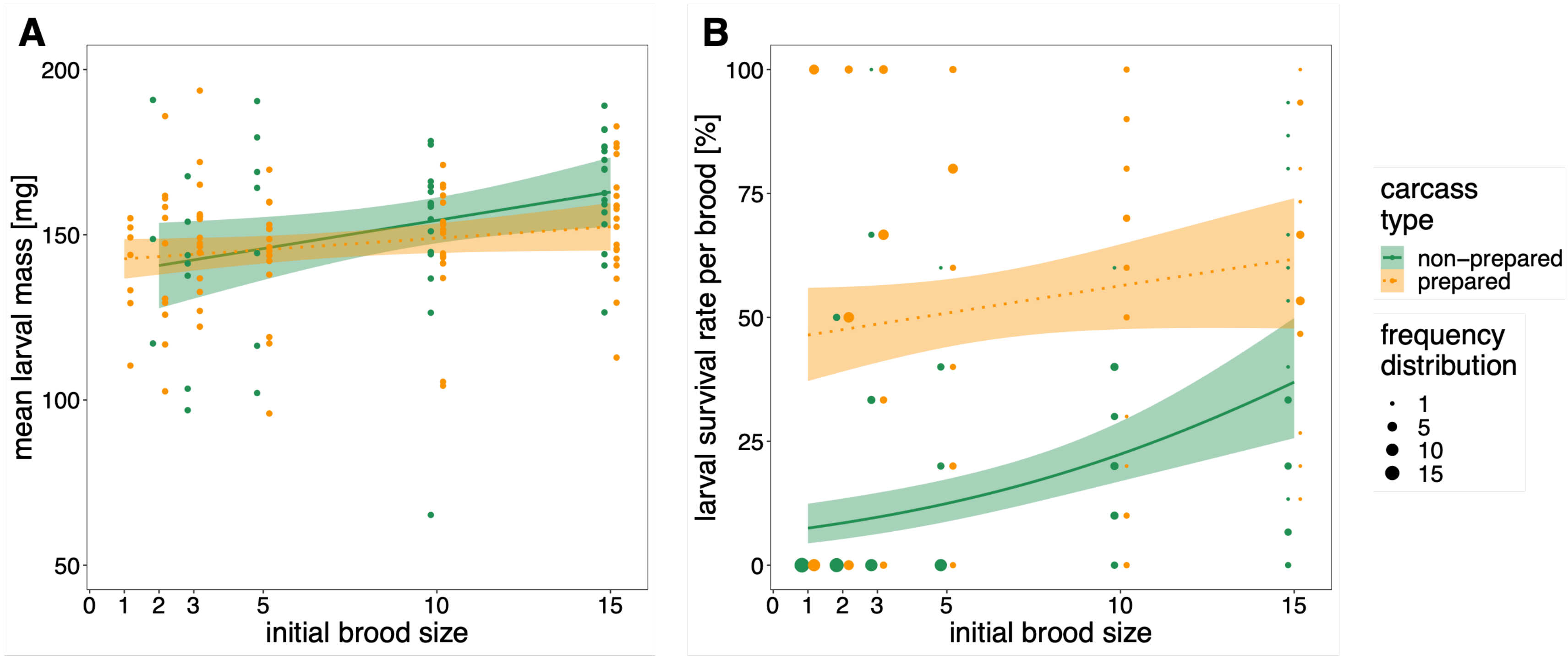

Figure 4.3
The relationship between initial brood size and two measures of larval performance in *N. vespilloides*: (A) the mean larval mass per brood at dispersal (n =138) and (B) the larval survival rate per brood until dispersal (n = 228). Larvae either developed on non-prepared carcasses or parentally prepared carcasses. The sizes of data points represent the number of broods with identical survival per brood in (B). The lines are smoothed regression lines and the shaded regions represent their 95 % confidence intervals. (©Figure adapted from (Prang *et al.* 2022))

Discussion

Recent studies have highlighted the importance of cooperative sibling interactions to the emergence and maintenance of family life. In this presumably early, non-derived 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 & 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.

We found evidence for sibling cooperation – i.e., 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, i.e., 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, i.e., 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 & 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 non-prepared carcasses, all three species showed a positive association of brood size with larval growth rate, while 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 non-prepared 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 hours. This is because we focused on larval cooperation in our study and therefore chose, based on the findings of Schrader *et al.* (2015a), 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, while 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, while less dependent larvae display high survival throughout, possibly masking any such effect. The provision of prehatching care improves larval performance (Eggert, Reinking & Müller 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, Plata*, et al.* 2018), (2) shift the carcasses' microbiome towards beneficial microbes (Duarte *et al.* 2018; Shukla, Vogel*, et al.* 2018), (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, Jarrett & Kilner 2015a), whereas benign conditions were shown to facilitate competition among larvae (Smiseth, Lennox & Moore 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 (*Lucilla sericata*), where benefits were shown to be mediated by thermal gains and exodigestion of carrion (Scanvion, Hédouin & Charabidzé 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. While not investigated in *Nicrophorus*, parentally derived biofilms were also shown to generate heat in *Necrodes littoralis* (Matuszewski & Mądra-Bielewicz 2021).

There are additional, non-mutually 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, Smiseth & Rozen 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 like *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 *et al.* 2021; Trumbo & Sikes 2021). However, decaying processes caused by carcass- and soil-borne microbes might act pre-digestive, facilitating self-feeding, especially among highly dependent species (Capodeanu-Nägler *et al.* 2016). Such effects might explain why in the first 24 hours, *N. orbicollis* survived better on non-prepared 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 & 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 like *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 byproduct, i.e., 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 non-prepared carcasses. Similarly, Schrader et al. (2015a) reported evidence for sibling cooperation in the presence of prehatching care - however, the prepared carcasses in their study might more closely resemble the non-prepared that we used. Since Schrader et al. (2015a) 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. (2015a) 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. (2015a) 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. S4.2** and **Table S4.1**). We found a positive effect of the number of dispersing offspring not only on non-prepared 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 *Ne. 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.

Supplementary Material

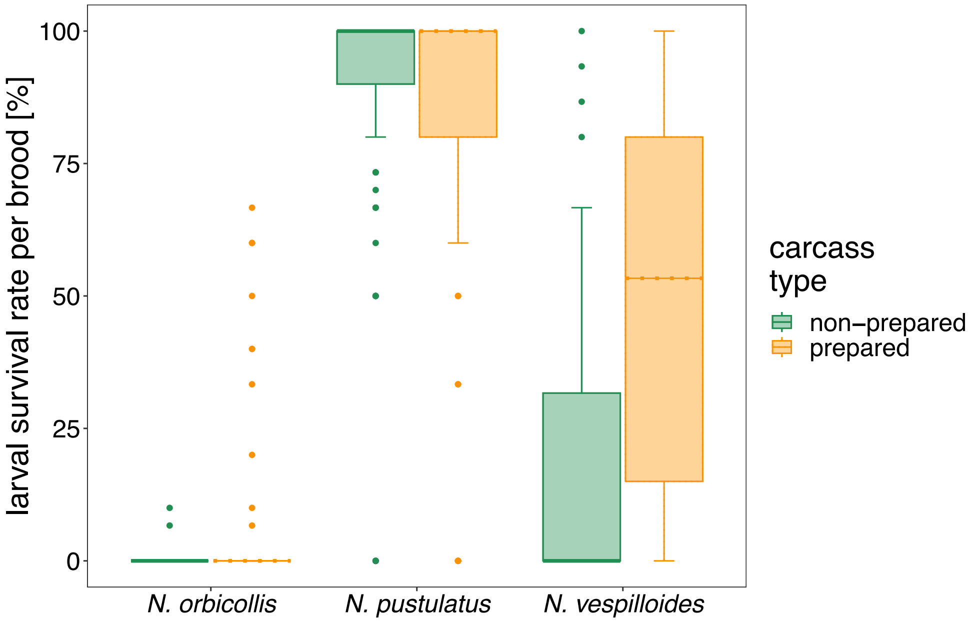

Figure S4.1

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). Larvae either developed on nonprepared carcasses or parentally prepared carcasses. Boxplots show the median, interquartile range and minimum/maximum range. The dots are values that fall outside the interquartile range (> 1.5 x interquartile range). (©Figure adapted from (Prang *et al.* 2022))

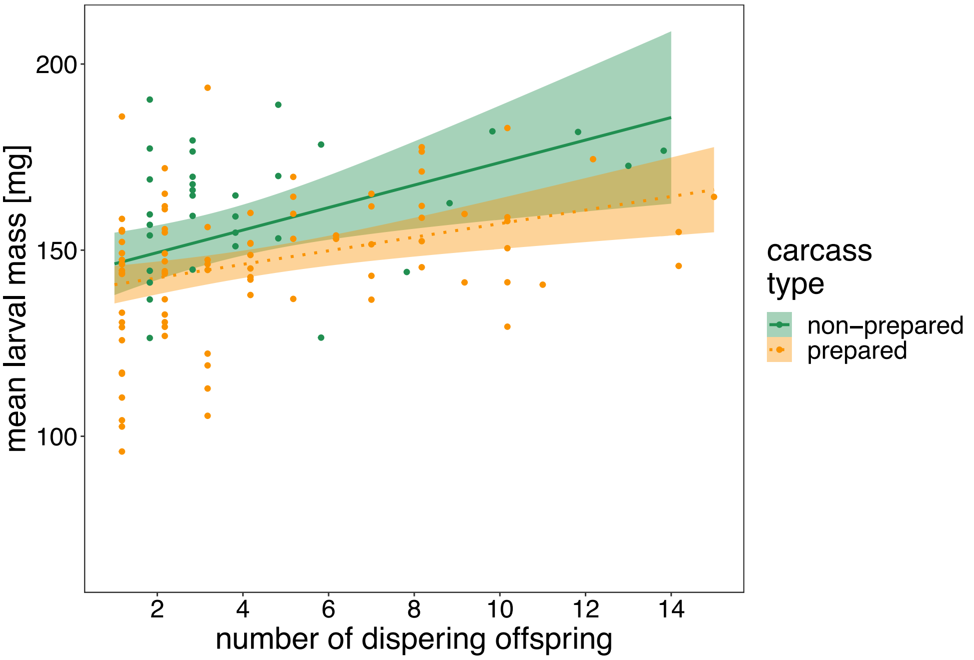

Figure S4.2

The relationship between the number of dispersing offspring and the mean larval mass per brood at dispersal in *N. vespilloides* (n = 228). Larvae either developed on nonprepared carcasses or parentally prepared carcasses. The lines are smoothed regression lines, and the shaded regions represent their 95 % confidence intervals. (©Figure adapted from (Prang *et al.* 2022))

Table S4.1

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. Larvae either developed on nonprepared or parentally prepared carcasses. The denominator degrees of freedom are indicated by df_d, while the numerator degrees of freedom were 1 in all cases. Each carcass type was analyzed separately. 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
Intercept	143.29	4.99	49	28.73	<0.001	138.90	2.95	87	47.01	<0.001
Number of dispersing larvae	3.02	1.07	48	2.84	**0.0067**	1.82	0.52	86	3.50	**<0.001**

References

Arce, A.N., Smiseth, P.T. & Rozen, D.E. (2013) Antimicrobial secretions and social immunity in larval burying beetles, *Nicrophorus vespilloides*. *Animal Behaviour,* 86, 741-745. https://doi.org/10.1016/j.anbehav.2013.07.008

Bell, M.B.V. (2007) Cooperative begging in banded mongoose pups. *Current Biology,* 17, 717-721. https://doi.org/10.1016/j.cub.2007.03.015

Botterill-James, T., Ford, L., While, G.M. & Smiseth, P.T. (2017) Resource availability, but not polyandry, influences sibling conflict in a burying beetle *Nicrophorus vespilloides*. *Behavioral Ecology,* 28, 1093-1100. https://doi.org/10.1093/beheco/arx073

Bowers, K.E., Sakaluk, S.K. & Thompson, C.F. (2013) Sibling cooperation influences the age of nest leaving in an altricial bird. *The American Naturalist,* 181, 775-786. https://doi.org/10.1086/670244

Capodeanu-Nägler, A., Eggert, A.-K., Vogel, H., Sakaluk, S.K. & Steiger, S. (2017) Species divergence in offspring begging and parental provisioning is linked to nutritional dependency. *Behavioral Ecology,* 29, 42-50. https://doi.org/10.1093/beheco/arx117

Capodeanu-Nägler, A., Keppner, E.M., Vogel, H., Ayasse, M., Eggert, A.-K., Sakaluk, S.K. & Steiger, S. (2016) From facultative to obligatory parental care: Interspecific variation in offspring dependency on post-hatching care in burying beetles. *Scientific Reports,* 6, 29323. https://doi.org/10.1038/srep29323

Capodeanu-Nägler, A., Prang, M.A., Trumbo, S.T., Vogel, H., Eggert, A.-K., Sakaluk, S.K. & Steiger, S. (2018) Offspring dependence on parental care and the role of parental transfer of oral fluids in burying beetles. *Frontiers in Zoology,* 15, 1-12. https://doi.org/10.1186/s12983-018-0278-5

Charabidze, D., Trumbo, S.T., Grzywacz, A., Costa, J.T., Benbow, M.E., Barton, P.S. & Matuszewski, S. (2021) Convergence of social strategies in carrion breeding insects. *BioScience,* 71, 1028-1037. https://doi.org/10.1093/biosci/biab068

Clutton-Brock, T.H. (1991) *The evolution of parental care*. Princeton University Press, Princeton, New Jersey, USA, https://doi.org/10.1046/j.1420-9101.1992.5040719.x

Costa, J.T. (2006) *The other insect societies*. Harvard University Press, Cambridge, Massachusetts, USA,

Cotter, S.C. & Kilner, R.M. (2010a) Personal immunity versus social immunity. *Behavioral Ecology,* 21, 663-668. https://doi.org/10.1093/beheco/arq070

Cremer, S., Armitage, S.A.O. & Schmid-Hempel, P. (2007) Social immunity. *Current Biology,* 17, R693-R702. https://doi.org/10.1016/j.cub.2007.06.008

Duarte, A., Welch, M., Swannack, C., Wagner, J. & Kilner, R.M. (2018) Strategies for managing rival bacterial communities: Lessons from burying beetles. *Journal of Animal Ecology,* 87, 414-427. https://doi.org/10.1111/1365-2656.12725

Eggert, A.-K. & Müller, J.K. (1997) Biparental care and social evolution in burying beetles: Lessons from the larder. *The evolution of social behavior in insects and arachnids* (eds J.C. Choe & B.J. Crespi), pp. 216-236. Cambridge, Massachusetts, U.S.A, https://doi.org/10.1017/CBO9780511721953.011

Eggert, A.-K., Reinking, M. & Müller, J.K. (1998) Parental care improves offspring survival and growth in burying beetles. *Animal Behaviour,* 55, 97-107. https://doi.org/10.1006/anbe.1997.0588

Falk, J., Wong, J.W.Y., Kölliker, M. & Meunier, J. (2014) Sibling cooperation in earwig families provides insights into the early evolution of social life. *The American Naturalist,* 183, 547-557. https://doi.org/10.1086/675364

Gruszka, J., Krystkowiak-Kowalska, M., Frątczak-Łagiewska, K., Mądra-Bielewicz, A., Charabidze, D. & Matuszewski, S. (2020) Patterns and mechanisms for larval aggregation in carrion beetle *Necrodes littoralis* (Coleoptera: Silphidae). *Animal Behaviour,* 162, 1-10. https://doi.org/10.1016/j.anbehav.2020.01.011

Hall, C.L., Wadsworth, N.K., Howard, D.R., Jennings, E.M., Farrell, L.D., Magnuson, T.S. & Smith, R.J. (2011) Inhibition of microorganisms on a carrion breeding resource: The antimicrobial peptide activity of burying beetle (Coleoptera: Silphidae) oral and anal secretions. *Environmental Entomology,* 40, 669-678. https://doi.org/10.1603/EN10137

Hamilton, C., Lejeune, B.T. & Rosengaus, R.B. (2011) Trophallaxis and prophylaxis: Social immunity in the carpenter ant *Camponotus pennsylvanicus*. *Biology Letters,* 7, 89-92. https://doi.org/10.1098/rsbl.2010.0466

Hamilton, W.D. (1964a) The genetical evolution of social behaviour. I. *Journal of Theoretical Biology,* 7, 1-16. https://doi.org/10.1016/0022-5193(64)90038-4

Hamilton, W.D. (1964b) The genetical evolution of social behaviour. II. *Journal of Theoretical Biology,* 7, 17-52. https://doi.org/10.1016/0022-5193(64)90039-6

He, S., Johnston, P.R., Kuropka, B., Lokatis, S., Weise, C., Plarre, R., Kunte, H.-J. & McMahon, D.P. (2018) Termite soldiers contribute to social immunity by synthesizing potent oral secretions. *Insect Molecular Biology,* 27, 564-576. https://doi.org/10.1111/imb.12499

Hoback, W.W., Bishop, A.A., Kroemer, J., Scalzitti, J. & Shaffer, J.J. (2004) Differences among antimicrobial properties of carrion beetle secretions reflect phylogeny and ecology. *Journal of Chemical Ecology,* 30, 719-729. https://doi.org/10.1023/b:joec.0000028427.53141.41

Kim, K.W., Krafft, B. & Choe, J.C. (2005) Cooperative prey capture by young subsocial spiders. *Behavioral Ecology and Sociobiology,* 59, 92-100. https://doi.org/10.1007/s00265-005-0013-z

Klug, H., Alonzo, S.H. & Bonsall, M.B. (2012) Theoretical foundations of parental care. *The evolution of parental care* (eds N.J. Royle, P.T. Smiseth & M. Kölliker), pp. 21-36. Oxford University Press, Oxford, U.K., https://doi.org/10.1093/acprof:oso/9780199692576.003.0002

Kramer, J. & Meunier, J. (2019) The other facets of family life and their role in the evolution of animal sociality. *Biological Reviews,* 94, 199-215. https://doi.org/10.1111/brv.12443

Kramer, J., Thesing, J. & Meunier, J. (2015) Negative association between parental care and sibling cooperation in earwigs: A new perspective on the early evolution of family life? *Journal of Evolutionary Biology,* 28, 1299-1308. https://doi.org/10.1111/jeb.12655

Magneville, C., Ratz, T., Richardson, J. & Smiseth, P.T. (2018) No evidence of sibling cooperation in the absence of parental care in *Nicrophorus vespilloides*. *Evolution,* 72, 2803-2809. https://doi.org/10.1111/evo.13622

Marti, C.D. (1989) Food sharing by sibling common barn-owls. *The Wilson Bulletin,* 101, 132-134.

Matuszewski, S. & Mądra-Bielewicz, A. (2021) Heat production in a feeding matrix formed on carrion by communally breeding beetles. *Frontiers in Zoology,* 18, 1-11. https://doi.org/10.1186/s12983-020-00385-7

Miller, C.J., Bates, S.T., Gielda, L.M. & Creighton, J.C. (2019) Examining transmission of gut bacteria to preserved carcass via anal secretions in *Nicrophorus defodiens*. *PLoS One,* 14, e0225711. https://doi.org/10.1371/journal.pone.0225711

Milne, L.J. & Milne, M. (1976) The social behavior of burying beetles. *Scientific American,* 235, 84-89. https://doi.org/10.1038/scientificamerican0876-84

Mock, D.W. & Parker, G.A. (1997) *The evolution of sibling rivalry*. Oxford University Press, New York, New York, USA, ISBN: 9780198577447

Parker, G.A. (1979) Sexual selection and sexual conflict. *Sexual selection and reproductive competition in insects* (eds M.S. Blum & N.A. Blum), pp. 123-166. Academic Press, INC., New York, U.S.A.,

Pukowski, E. (1933) Ökologische Untersuchungen an *Necrophorus* F. *Zeitschrift für Morphologie und Ökologie der Tiere,* 27, 518-586. https://doi.org/10.1007/BF00403155

Pukowski, E. (1934a) Die Brutpflege des Totengräbers. *Entomologische Blätter,* 30, 109-113.

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

Rebar, D., Bailey, N.W., Jarrett, B.J.M. & Kilner, R.M. (2020) An evolutionary switch from sibling rivalry to sibling cooperation, caused by a sustained loss of parental care. *Proceedings of the National Academy of Sciences,* 117, 2544-2550. https://doi.org/10.1073/pnas.1911677117

Royle, N.J., Smiseth, P.T. & Kölliker, M. (2012) *The evolution of parental care*. Oxford University Press, Oxford, U.K., https://doi.org/10.1093/acprof:oso/9780199692576.001.0001

Scanvion, Q., Hédouin, V. & Charabidzé, D. (2018) Collective exodigestion favours blow fly colonization and development on fresh carcasses. *Animal Behaviour,* 141, 221-232. https://doi.org/10.1016/j.anbehav.2018.05.012

Schrader, M., Jarrett, B.J. & Kilner, R.M. (2015a) Parental care masks a density-dependent shift from cooperation to competition among burying beetle larvae. *Evolution,* 69, 1077-1084. https://doi.org/10.1111/evo.12615

Shukla, S.P., Plata, C., Reichelt, M., Steiger, S., Heckel, D.G., Kaltenpoth, M., Vilcinskas, A. & Vogel, H. (2018) Microbiome-assisted carrion preservation aids larval development in a burying beetle. *Proceedings of the National Academy of Sciences,* 115, 11274-11279. https://doi.org/10.1073/pnas.1812808115

Shukla, S.P., Vogel, H., Heckel, D.G., Vilcinskas, A. & Kaltenpoth, M. (2018) Burying beetles regulate the microbiome of carcasses and use it to transmit a core microbiota to their offspring. *Molecular Ecology,* 27, 1980-1991. https://doi.org/10.1111/mec.14269

Smiseth, P.T., Darwell, C.T. & Moore, A.J. (2003) Partial begging: An empirical model for the early evolution of offspring signalling. *Proceedings of the Royal Society B: Biological Sciences,* 270, 1773-1777. https://doi.org/10.1098/rspb.2003.2444

Smiseth, P.T., Lennox, L. & Moore, A.J. (2007) Interaction between parental care and sibling competition: Parents enhance offspring growth and exacerbate sibling competition. *Evolution,* 61, 2331-2339. https://doi.org/10.1111/j.1558-5646.2007.00192.x

Smiseth, P.T. & Moore, A.J. (2002) Does resource availability affect offspring begging and parental provisioning in a partially begging species? *Animal Behaviour,* 63, 577-585. https://doi.org/10.1006/anbe.2001.1944

Stow, A., Briscoe, D., Gillings, M., Holley, M., Smith, S., Leys, R., Silberbauer, T., Turnbull, C. & Beattie, A. (2007) Antimicrobial defences increase with sociality in bees. *Biology Letters,* 3, 422-424. https://doi.org/10.1098/rsbl.2007.0178

Suzuki, S. (1999) Does carrion-burial by *Nicrophorus vespilloides* (Silphidae: Coleoptera) prevent discovery by other burying beetles? *Entomological Science,* 2, 205-208. ISSN: 13438786

Trivers, R.L. (1974) Parent-offspring conflict. *American Zoologist,* 14, 249-264. https://doi.org/10.1093/icb/14.1.249

Trumbo, S.T. (1992) Monogamy to communal breeding: Exploitation of a broad resource base by burying beetles (*Nicrophorus*). *Ecological Entomology,* 17, 289-298. https://doi.org/10.1111/j.1365-2311.1992.tb01060.x

Trumbo, S.T., Philbrick, P.K.B., Stökl, J. & Steiger, S. (2021) Burying beetle parents adaptively manipulate information broadcast from a microbial community. *The American Naturalist,* 197, 366-378. https://doi.org/10.1086/712602

Trumbo, S.T. & Sikes, D.S. (2021) Resource concealment and the evolution of parental care in burying beetles. *Journal of Zoology,* 315, 175-182. https://doi.org/10.1111/jzo.12916

Vogel, H., Shukla, S.P., Engl, T., Weiss, B., Fischer, R., Steiger, S., Heckel, D.G., Kaltenpoth, M. & Vilcinskas, A. (2017) The digestive and defensive basis of carcass utilization by the burying beetle and its microbiota. *Nature Communications,* 8, 1-15. https://doi.org/10.1038/ncomms15186

Wang, Y. & Rozen, D.E. (2018) Gut microbiota in the burying beetle, *Nicrophorus vespilloides*, provide colonization resistance against larval bacterial pathogens. *Ecology and Evolution,* 8, 1646-1654. https://doi.org/10.1002/ece3.3589

West, S.A., Griffin, A.S. & Gardner, A. (2007a) Evolutionary explanations for cooperation. *Current Biology,* 17, R661-R672. https://doi.org/10.1016/j.cub.2007.06.004

West, S.A., Griffin, A.S. & Gardner, A. (2007b) Social semantics: Altruism, cooperation, mutualism, strong reciprocity and group selection. *Journal of Evolutionary Biology,* 20, 415-432. https://doi.org/10.1111/j.1420-9101.2006.01258.x

Wong, J.W.Y., Meunier, J. & Kölliker, M. (2013) The evolution of parental care in insects: The roles of ecology, life history and the social environment. *Ecological Entomology,* 38, 123-137. https://doi.org/10.1111/een.12000

Yip, E.C. & Rayor, L.S. (2013) The influence of siblings on body condition in a social spider: Is prey sharing cooperation or competition? *Animal Behaviour,* 85, 1161-1168. https://doi.org/10.1016/j.anbehav.2013.03.016

Ziadie, M.A., Ebot-Ojong, F., McKinney, E.C. & Moore, A.J. (2019) Evolution of personal and social immunity in the context of parental care. *The American Naturalist,* 193, 296-308. https://doi.org/10.1086/701122

Publication Record

Record of all publications presented in this dissertation

Prang, M. A., Zywucki, L., Körner, M., & Steiger, S. (2022). Differences in sibling cooperation in presence and absence of parental care in a genus with interspecific variation in offspring dependence. *Evolution*, *76*(2), 320-331. https://doi.org/10.1111/evo.14414

Capodeanu-Nägler, A., **Prang, M. A.**, Trumbo, S. T., Vogel, H., Eggert, A. K., Sakaluk, S. K., & Steiger, S. (2018). Offspring dependence on parental care and the role of parental transfer of oral fluids in burying beetles. *Frontiers in Zoology*, *15*(1), 1-12. https://doi.org/10.1186/s12983-018-0278-5

Record of further own publications not used in this dissertation

Keppner, E. M., Laubenthal, M., **Prang, M. A.**, Conrad, T., & Steiger, S. (2023). Harsh nutritional environment has positive and negative consequences for family living in a burying beetle. *Ecology and Evolution*, *13*(1), e9699. https://doi.org/10.1002/ece3.9699

Sahm, J., **Prang, M. A.**, & Steiger, S. (2022). Parent–offspring conflict and its outcome under uni- and biparental care. *Scientific Reports*, *12*(1), 1999 https://doi.org/10.1038/s41598-022-05877-6

Keppner, E. M., **Prang, M. A.**, Engel, K. C., Ayasse, M., Stökl, J., & Steiger, S. (2017). Beyond cuticular hydrocarbons: chemically mediated mate recognition in the subsocial burying beetle *Nicrophorus vespilloides*. *Journal of Chemical Ecology*, *43*, 84-93. https://doi.org/10.1007/s10886-016-0806-8

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Statutory declarations

(Eidesstattliche) Versicherungen und Erklärungen

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