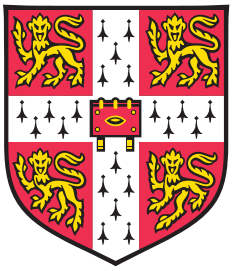
BENJAMIN JAMES MERVYN JARRETT

THE ROLE OF PARENTS IN EVOLUTION



The Role of Parents in Evolution

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Submitted for the degree of Doctor of Philosophy

University of Cambridge, September 2017

Supervisor:
Prof. Rebecca Kilner

DECLARATION

This dissertation is submitted for the degree of Doctor of Philosophy. It is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text and acknowledgments. It is not substantially the same as any previous material that I have submitted, or, that is being concurrently submitted for a degree, diploma or other qualification at the University of Cambridge, any other University, or similar institution. This thesis does not exceed the prescribed word limit.

Cambridge, September 2017

Benjamin James Mervyn Jarrett

ABSTRACT

In this thesis, I investigated the role of parental care in evolution. Parents provide the environment in which offspring develop and therefore have a large influence on their offspring's phenotypes, and so are in prime position to influence evolutionary processes. I used an experimental approach, and focused on the burying beetle, *Nicrophorus vespilloides*. The burying beetle is a perfect system for this question: they exhibit elaborate biparental care which is correlated with rapid speciation in the *Nicrophorus* genus.

I started with a thorough exploration of burying beetle ecology and how the guild structure and interspecific competition in local populations can shape phenotypic evolution of my focal species, *N. vespilloides*. Interspecific competition shapes how the carrion niche is partitioned, which feeds back onto the evolution of body size within *Nicrophorus* reducing competition. The evolution of parental care in this genus likely facilitated its adaptive radiation, as parental care is linked with body size, both within and across species.

But to what extent does the ecology shape the production and maintenance of phenotypic and genetic variation? I then use a quantitative genetic approach to show that body size and development time of *N. vespilloides* shows no additive genetic variation. Evolution of these fitness related traits can only occur through maternal effects or sibling effects.

I tested this prediction by mimicking the radiation of the burying beetles by imposing my own selection on body size when parents could care for their offspring and when they could not. The presence of post-hatching parental care dramatically changed how populations responded to selection, through a combination of cooperation between parents and offspring, and cooperation between offspring.

As well as shaping the evolutionary potential of populations, an experimental change in parental care can induce new selective forces, favouring adaptive novelties for the new social environment. Larvae evolving without parental care evolved disproportionately larger mandibles when small to better adapt them to a life without care.

Much is known about the evolution of parental care across the animal kingdom, but what happens next—are the burying beetles a "one-off"? I compiled data across the arthropods comparing clades that exhibit post-hatching parental care with their sister clades and show that clades with care are more species rich. While the mechanism may not be the same as with *Nicrophorus*, I discussed other potential mechanisms that may be at play in the role of parents in evolution.

ACKNOWLEDGMENTS

I firstly thank my supervisor Becky Kilner for her brilliant and wonderful supervision, support, and insight. I am confident in that I would not have wanted to do my PhD under anyone else.

Secondly, I thank Matt Schrader, with whom I have spent a great deal of time in the lab. In that time I learnt a great deal of what it takes to be a scientist and how to approach my own work, for which I am most grateful. It was a real joy spending too long in a windowless basement looking at beetles with you. A big thank you to Darren Rebar too, for many beers and tangential conversations about most things while we looked at beetles in a slightly bigger windowless room.

A great big thank you to the other members of the burying beetle group that kept me sane and happy throughout: Ana Duarte for being so kind and welcoming, and for your sharp mind; to Ornela de Gasperin, for your help in getting faster at lab work and your always chipper outlook (so long as it was sunny); to Amy Backhouse who welcomed me so generously and set me on my way; to Alfredo Attisano for you field work and attitude to food; to Syuan-Jyun Sun for your friendship in the lab and help with pretty much everything; to Swastika Issar for help with the experiment that did not make it into this thesis; to Sonia for your consistency and wise words; to Rahia Mashoodh for conversations about plasticity and epigenetics, which bamboozled me; to Chris Swannack for your calm presence and aid with all things computery; and to Sue Aspinall for being the most splendid and dependable of lab mates. Thanks too, to Laura Bampton, Emmanuelle Briolat, Andrew Catherall, Emma Evans, Hannah Haynes, Miranda Leaf, Timo Rossberg, and all the other Part II or part-time helpers in the lab, of which this is an incomplete list I am sure.

Thank you to the wonderful European Research Council, who funded me and the research in this thesis. Collaboration across the EU and the world is incredible important and valuable. My research, and research in the UK, would not be where it is without EU initiatives like the ERC.

A large thank you to Santiago Castañón, who wrote the MATLAB code upon which most of this thesis rested. For Chapter 3, I thank Will Pearse for feedback and help with the analysis, Kiyoko Gotanda for comments on the chapter, Marius Somveille for aid with ArcGIS, Max Barclay and Roger Booth for access to the Coleoptera collection at the Natural History Museum, Daniel Howard and Curtis Creighton for information about parental care in other burying beetles, and Alfredo Attisano and Syuan-Jyun Sun for field data. For Chapter 4, I thank Thomas Houslay for help analysing the data and comments on the chapter, and Darren Rebar for help collecting the data. For Chapter 5, I thank Sue Aspinall, Alfredo Attisano, Emmanuelle Briolat, Ana Duarte, Ornela de Gasperin, Becky Kilner, Matt Schrader, and Chris Swannack for help in the lab to collect data. For Chapter 6, I thank Emma Evans, William Haynes, and Miranda Leaf for help collecting the experimental data, Ana Duarte and Darren Rebar for collecting the pre-hatching feeding hole data, Keturah Smithson and Rob Asher for assistance and help CT scanning larval mandibles, and David Labonte and Ian Warren for comments.

I would also like to thank my advisors Chris Jiggins and Nick Davies for their crucial comments and questions during the development of this thesis. It was a pleasure having an office opposite Nick's, if just to have him poke his head in occasionally and encourage me, or, more frequently, mention the birds he has seen that weekend.

Thank you to Rose Thorogood for being a fantastic first office mate and showing me the ropes. And to Claire Spottiswoode who has been so kind and willing to listen to most any old rubbish that I spout—thank you. And there is big money in it when I pass…

I owe a large thank you to the rest of the Behavioural Ecology group, who have sat through a small number of my meetings and contributed a large dose of help and friendship in my time here. In alphabetical order, I thank Neeltje Boogert, Mike Brooke, Alecia Carter, Dom Cram, Teja Chalikonda, Dave Daversa, Elisa Dierickx, Constance Dubuc, Jenny Easley, Sinead English, Vix Franks, Kiyoko Gotanda, Liisa Hämäläinen, Nick Horrocks, Gabriel Jamie, Arne Jungwirth, Helen Leggett, Corina Logan, Dieter Lukas, Kirsty MacLeod, Raff Mares, Katie McGhee, Antonio Rodrigues, Hannah Rowland, Marj Sorensen, Jack Thorley, Wenfei Tong, Philippe Vullioud, Leila Walker, Peter Woodford, Jenny York, and Markus Zöttl.

It was a great joy doing my PhD in the Zoology department surrounded by such wonderful and friendly staff. A warm thank you to all with whom I have played and captained in the Zootallurgy Cricket Team. We went through some lows, but such lofty heights.

The Thesis Writing Club helped a great deal in constructing this piece of work. Gabriel Jamie and Elisa Dierickx were fantastic support during the writing process, with additional support from part-time members Jack Thorley, Vix Franks, Anne-Sophie Bonnet-Lebrun, and Teja Chalikonda.

A massive thank you to the friends off whom I have bounced ideas. My thoughts about evolutionary genetics are what they are through chats with Simon Martin, and it was always comforting having Simon opening the batting for the Zoots. My knowledge and increasing respect of quantitative genetics is due only to Tom Houslay. Thank you Tom, for the beach beers and the patience of explaining to me how to decompose phenotypic variation for the umpteenth time. And in all things morphological and biomechanical, I pick the brain of David Labonte over plenty of good quality alcohol and fantastic food. Thank you.

A very special thank you to Kirsty MacLeod, who has seen this thesis from the start to the end. And suffered my company the whole way too. Kirsty has seen me through all the experiments and beetle-induced pain with encouragement and love. Thank you so very much.

Lastly, it is hard to do a PhD on parental care and family life and not acknowledge the large influence my own family has had on me. Thank you to Thomas, Sam, and Jonathan, who taught me everything I need to know about sibling conflict and cooperation. And to my parents, Richard and Louise, I thank with everything I have.

PUBLICATIONS

Below is a list of all the papers resulting directly and indirectly from my PhD work. They can all be found as PDFs in the Appendix.

Schrader M, **Jarrett BJM**, Rebar D & Kilner RM (2017) Adaptation to a novel family environment involves both apparent and cryptic phenotypic changes *Proceedings of the Royal Society B: Biological Sciences* **284** 20171295

Jarrett BJM, Schrader M, Rebar D, Houslay TM & Kilner RM (2017) Cooperative interactions within the family enhance the capacity for evolutionary change in body size. *Nature Ecology & Evolution* **1** 0178

Schrader M, Crosby RM, Hesketh AR, **Jarrett BJM** & Kilner RM (2016) A limit to the extent to which increased egg size can compensate for a poor post-natal environment, revealed experimentally in the burying beetle, *Nicrophorus vespilloides*. *Ecology & Evolution* **6** 329–336

Kilner RM, Boncoraglio G, Henshaw J, **Jarrett BJM**, De Gasperin O, Attisano A & Kokko H (2015) Parental effects alter the adaptive value of an adult behavioural trait. *eLife* 10.7554

Schrader M, **Jarrett BJM** & Kilner RM (2015) Using experimental evolution to study adaptations for life within the family. *The American Naturalist* **185** 610–619

Schrader M, **Jarrett BJM** & Kilner RM (2015) Parental care masks a density-dependent shift from mutually beneficial to competitive sibling interactions in burying beetle broods. *Evolution* **69** 1077–1084

Also included in the Appendix is a manuscript currently in preparation.

Pascoal S, **Jarrett BJM**, Evans E & Kilner RM. Superior stimulation of female fecundity by subordinate males provides a mechanism for telegony.

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Part I

INTRODUCTION

1

INTRODUCTION

"Natural selection will modify the structure of the young in relation to the parent, and of the parent in relation to the young."
— Charles Darwin (1859) *On the origin of species*

1.1 THE EVOLUTION OF PARENTAL CARE

Parental care exists in diverse and extraordinary forms across the animal kingdom. Any behaviour expressed in the parent that increases their offspring's fitness is defined as parental care (Clutton-Brock, 1991; Royle, Smiseth and Kölliker, 2012). Care by parents can consist of pre-hatching care, like the selection of oviposition sites that reduce offspring mortality from predators (e. g. Spencer, Blaustein and Cohen, 2002), and the guarding and tending of eggs to prevent predation or decay (Boos et al., 2014). The construction of nests or burrows is also a form of pre-hatching parental care in that offspring are sheltered throughout development from predation and parasitism, as well as from environmental extremes like flooding or desiccation (Gilbert, 2014; Wyatt, 1986). Nests and burrows can also act as larders where parents may leave a cache of resources like paralysed insects or dung, for example, on which offspring will feed (Hanski and Cambefort, 1991). Parents also provision their offspring with food, which can occur before birth in viviparous species (Blackburn, 2006; Meier, Kotrba and Ferrar, 1999), as well as post-birth. It might involve the supply of paralysed insects in *Ammophila* wasps (Field, 1992), pre-digested carrion in *Nicrophorus* burying beetles (Scott, 1998b), or the mother herself, sacrificed to be consumed by her offspring (Evans, Wallis and Elgar, 1995; Yip and Rayor, 2014).

How and why parental care in this diversity of forms (Figure 1.1) has evolved has a long history of empirical and theoretical work (Clutton-Brock, 1991; Cockburn, 2006; Gilbert and Manica, 2015; Klug and Bonsall, 2010; Kutschera and Wirtz, 2001; Royle, Smiseth and Kölliker, 2012; Tallamy and Wood, 1986; Thiel, 2000; Wong, Meunier and Kölliker, 2013). Certain ecological conditions favour the evolution of post-hatching parental care; for example, when resources have a patchy spatial distribution or are ephemeral. In these cases, parents need to find and guard resources in order to feed their young, thus selecting for parental care (Brown, Morales and Summers, 2010; Gardner and Smiseth, 2011). Some resources, like detritus or dead wood, are plentiful in contrast, but are hard to

Figure 1.1 | Some examples of parental care in the animal world. Anemonefish guard their offspring after they hatch (top left, photograph by Silke Baron). Mimic poison frog (*Ranitomeya imitator*) transporting a tadpole (top right, photograph by John Clare). Spiders also transport their offspring (bottom left, photographed by Thomas Houslay), but mothers are often their feeding resource too. Burying beetles (*Nicrophorus vespilloides*) provision their offspring as they feed on a dead mouse (bottom right, photograph by Thomas Houslay).

digest and extract nutrients from. Parental care in termites and bark beetles has also evolved to aid digestion of these resources, which requires the faithful transmission of symbionts from mother to offspring (Tallamy and Wood, 1986). Provisioning offspring can also evolve as a consequence of greater predation or parasitism risk. Guarding eggs and offspring by parents reduces their mortality (Tallamy and Denno, 1981), and also serves as a pre-adaptation for the evolution of provisioning behaviour (Field, 2005; Field and Brace, 2004).

Certain life history traits can also shape the evolution of extended parental care (Clutton-Brock, 1991; Gilbert and Manica, 2010; Klug, Alonzo and Bonsall, 2012; Klug and Bonsall, 2010; Stearns, 1976). For offspring and parents to interact post-hatching, they must both reside in the same geographical space, and parents should live long enough to be able to interact with their young (Tallamy and Wood, 1986). Ultimately, it is the interplay between the life history and the ecology of a species that determines the costs of rearing more than one brood, and therefore the benefits to investing in a single brood through the evolutionary elaboration of parental care. Evolutionary patterns of egg size and clutch size across insects, for example, show that species that exhibit parental care have smaller clutches (Gilbert and Manica, 2010), and parental care and life history evolution go hand in hand. Such a pattern is correlational, however. Parental care may have evolved in response to small clutches in order to maximise offspring

survival, or the evolution of parental care subsequently shaped the evolution of clutch size.

The relationship between parental care and clutch size is one example where the potential role of parental care in evolution is not causally known—but there are many more. How parental interactions with offspring guide and change the way selection acts, and the way that evolution unfolds, is not well understood and demands testing through experimentation. There are two non-mutually exclusive mechanisms that are implicated: maternal effects and social selection. The role of both maternal effects and social selection in the evolutionary process have been considered in some depth and so offer theory and data on which to base predictions. But neither has been considered explicitly in the context of animal families, nor been used to investigate the role of parental care in evolution.

1.2 MATERNAL EFFECTS

Parents affect their offspring's phenotype first through their genetic contributions, and secondly through non-genetic contributions, like the environment they provide or epigenetic marks and influences (Weaver et al., 2004; Wolf and Wade, 2009). Wolf and Wade (2009) define a maternal effect as *"the* causal *influence of the maternal genotype or phenotype on the offspring phenotype"*, which includes parental care. While parental care has seldom been explicitly considered as a driving force in evolution, maternal effects are considered to have a large influence on ecological and evolutionary processes.

Maternal effects can have a huge influence on offspring phenotype and fitness, by altering the developmental environment their offspring will experience, or by adaptively matching their offspring's phenotype to the environment (Mousseau and Fox, 1998). For example, moor frog (*Rana arvalis*) mothers in more acidic environments lay eggs with a gelatinous capsule more resistant to acidic conditions (Räsänen, Laurila and Merilä, 2003), while maternal American bellflowers (*Campanulastrum americanum*) adaptively shape their offspring to have greater fitness in the same light environment they themselves developed in (Galloway and Etterson, 2007). A meta-analysis suggests, however, that these transgenerational adaptive maternal effects are not common (Uller, Nakagawa and English, 2013). Maternal effects also offer an alternative route to achieve adaptive phenotypes during development (Badyaev and Uller, 2009). Increased offspring growth in North American red squirrels (*Tamiasciurus hudsonicus*) is adaptively triggered by a change in squirrel density and mediated through maternal effects (Dantzer et al., 2013). The hatching order of western bluebird chicks (*Sialia mexicana*) is negatively correlated with aggression in male offspring, where first laid males are more aggressive than last laid males (Duckworth, 2009). This maternal effect

influences the cycles of recolonisation that occur in the mountain and western bluebirds. It changes ecological processes (Duckworth, Belloni and Anderson, 2015), and therefore indirectly influences evolution.

As well as influencing ecological processes, maternal effects also directly change and shape evolutionary trajectories (Badyaev and Uller, 2009; Räsänen and Kruuk, 2007). Maternal effects have been shown with theoretical work to change the response to selection (Falconer, 1965; Kirkpatrick and Lande, 1989; Lande and Kirkpatrick, 1990; McGlothlin and Galloway, 2014; Riska, 1989), which has been followed with empirical work confirming such effects (Badyaev et al., 2002; McAdam and Boutin, 2004; Rossiter, 1996; Wilson et al., 2005). Variation in maternal effects could also be accountable for potential divergence between populations, as is the case with house sparrows (*Carpodacus mexicanus*, Badyaev, Hill and Whittingham, 2002; Badyaev et al., 2002). In two populations of the house sparrow, the optimal laying order with respect to sex differs (Badyaev et al., 2003), which has led to rapid, differential evolution of growth rates between the sexes and subsequent sexual size dimorphism (Badyaev et al., 2002). In the house sparrow at least, the traits that have evolved most rapidly between the two populations are the traits most influenced by maternal effects (Badyaev, 2005). Maternal effects can therefore influence evolution by increasing the evolutionary potential of traits (McFarlane et al., 2015), as well as inducing evolutionary novelty (Badyaev, 2008; Badyaev and Uller, 2009).

1.3 SOCIAL SELECTION AND INDIRECT GENETIC EFFECTS

Social interactions have profound implications for evolution (West-Eberhard, 1979, 1983; Wolf, 2003). One way in which this can occur is through social selection, where the social environment directly exerts selection on conspecifics (Trubenová and Hager, 2012, 2014). For example, alloparental care behaviour in one individual directly affects the fitness of other group members (Riedman, 1982). Social selection, therefore, may be particular important in animal families, where parents interact with their offspring, and siblings interact with one another, and so reciprocally influence the nature and strength of selection acting on each other (West-Eberhard, 1979, 1983). The idea of social interactions leading to divergence and speciation was formulated by West-Eberhard (1979, 1983) in reference to interactions in a sexual selection context, where individuals compete for mates and resources associated with greater matings. West-Eberhard (1979) states that in scenarios without a social interaction, like that of a changed temperature, natural selection will favour adaptation to a point; to "*… a degree of refinement where further improvement would yield such greatly diminished returns that selection would not produce further marked change*". She goes on to say that in

sexual selection (or, more broadly, whenever social interactions occur), a change in competitive ability is always favoured unless through selection in another context, which changes the environment in a way a change of temperature does not, so selection can always act on individuals to pass the new bench mark. This is because *"... conspecific rivals are an environmental contingency that can itself evolve"* (West-Eberhard, 1979). It is this train of thought—of individuals comprising of not just a component of the environment, but a component of the environment that can *evolve*—that led to the theory of indirect genetic effects (IGEs, McGlothlin et al., 2010; Moore, Brodie and Wolf, 1997; Wolf, Brodie and Moore, 1999).

The theory of IGEs expanded the ideas of West-Eberhard (1979), combining it with Griffing's (1967) idea of associative effects, to include any social interaction occurs between individuals. An IGE exists when the genotype of one individual influences the phenotype of another individual, with whom they are interacting. The phenotype of one individual is therefore not just governed by their own genes and the environment they experience, but also by the genes within their social environment. Since the social environment consists of genes, it can evolve and in turn affect the evolution of phenotypes of their social partners. IGE theory predicts that social interactions can accelerate or retard evolutionary change, depending on the direction and magnitude of the interaction (McGlothlin et al., 2010; Moore, Brodie and Wolf, 1997; Wolf, Brodie and Moore, 1999). If the interaction is large and positive with respect to the direction of selection, feedback loops positively reinforce change across generations (Bailey and Zuk, 2012; Bleakley and Brodie, 2009; Chenoweth, Rundle and Blows, 2010). But when the interaction is negative with regards to the direction of selection, it impedes evolutionary change (Bijma, 2014; Bijma and Wade, 2008; Drown and Wade, 2014; Hadfield, 2012; Kirkpatrick and Lande, 1989; McGlothlin et al., 2010; Moore, Brodie and Wolf, 1997; Wade et al., 2010; Wolf, Brodie and Moore, 1999). Moreover, the nature of the social interaction can also evolve (Chenoweth, Rundle and Blows, 2010; Kazancíoğlu, Klug and Alonzo, 2012; Moore et al., 2002). Social interactions, especially within the family, have dynamic properties that change how natural selection operates on phenotypes (Hadfield and Thomson, 2017; Thomson and Hadfield, 2017), but also how populations respond to selection (Westneat, 2012).

1.4 THESIS PLAN

Through the combination of maternal effects, social selection, and IGEs within animal families, parents are likely to influence the evolutionary process. Whilst there is a considerable literature consistent with this suggestion, experimental evidence is lacking. In this thesis, I ask whether parental care accelerates the rate

of evolutionary change. I use information and theory from both the maternal effects and IGE literature to inform experimental work to better understand the evolutionary consequences of parental care. In Chapter 2, I begin by choosing an appropriate study organism: the burying beetle *Nicrophorus vespilloides*. I detail aspects of the life history of *Nicrophorus* burying beetles generally, as well as noting the appropriateness of the genus for the questions I am interested in asking. I end with a detailed outline of the methods used in the laboratory for maintaining the *N. vespilloides* populations.

In Chapter 3, I consider how interspecific competition for carrion, and body size evolution within the genus, has contributed to the adaptive radiation of *Nicrophorus*. In Chapter 4, I use a quantitative genetic approach to ask how genetic variation and phenotypic variation changes across social environments, to understand the evolutionary potential of parental care. I build on the results of Chapter 4 in Chapter 5, where I ask if parents can change the response to artificial selection for body size. In Chapter 6 I investigate an adaptive novelty in larvae that has evolved in response to a change in the social environment created by their parents. Lastly, in Chapter 7 I tie the thesis together by using a literature review of provisioning invertebrates to analyse whether parental provisioning could have a role in driving macroevolutionary patterns of diversification.

2

THE BURYING BEETLE

2.1 THE NATURAL HISTORY OF BURYING BEETLES

Burying beetles, or sexton beetles, comprise all 68 species in the genus *Nicrophorus*, and are found across the whole Northern Hemisphere and down the spines of mountain ranges stretching into the Southern Hemisphere (Sikes, Madge and Newton, 2002; Sikes and Venables, 2013). Burying beetles attracted the attention of many early naturalists, such as Jean-Henri Fabre (1918), as they exhibit the remarkably complex behaviour of extended biparental care. Burying beetles use small vertebrate carcasses as a breeding resource. The carcass of small mammals, birds, or fish, provide the sole resource for the parents to rear a whole brood. Vertebrate carcasses are likely unpredictable, ephemeral resources. Therefore parents must defend this valuable resource from inter- and intraspecific competitors, which likely led to the evolution of extended parental care in *Nicrophorus* (Pukowski, 1933; Scott, 1998b).

Upon finding a suitable carcass, adult burying beetles start to bury it by digging underneath it. Whilst burying occurs, both male and female shape the carcass into a ball and remove the hair or feathers with their mandibles (De Gasperin et al., 2016; Pukowski, 1933; Scott, 1998b). During this stage, the beetles make an incision into the carcass and extract and eat the gut of the carcass, presumably to prevent putrefaction from the intestinal bacteria of the carcass (Duarte et al., 2017). After this process, the adult beetles cover the carcass in anal exudate, which has antimicrobial properties, partly through lysozyme action (Cotter and Kilner, 2010; Cotter et al., 2010; Palmer et al., 2016). The anal exudate and the bacteria from the vertebrate gut change the bacterial community on the surface of the flesh (Duarte et al., 2017).

Whilst the carcass is being prepared, the female lays eggs in the soil around the grave, which, at the earliest, can start hatching approximately 59 hours after they were laid (Smiseth, Ward and Moore, 2006). The newly hatched larvae crawl towards the carcass and congregate in a small feeding depression made by the parents just prior to, or at, larval hatching. There, the larvae self-feed but are also fed by their parents (Smiseth and Moore, 2002b). Larvae beg to their parents using tactile signals who then regurgitate fluids to the larvae. Larvae also contribute to the social immunity by producing antimicrobial exudate (Arce, Smiseth and Rozen, 2013; Reavey, Beare and Cotter, 2014).

Once the carcass has been fully consumed, or the larvae reach a critical mass, they disperse from the carcass into the soil nearby. There, the larvae create a pupal chamber and develop into the adult. For the majority of species, there is one generation a year (univoltine), though for some of the smaller species like *N. vespilloides* it is thought there are two generations a year (bivoltine, Scott, 1998b). While following individuals in the wild presents difficulties, in the lab adults can successfully rear up to five consecutive broods if provided carcasses, indicating the capacity and likelihood that multiple breeding attempts do occur.

Figure 2.1 | The life stages of a burying beetle, *Nicrophorus vespilloides*. The generation time of *N. vespilloides* is approximately six weeks in the lab. Once presented with a suitable breeding resource, females start laying eggs which on average start hatching approximately 80 hours later. The larvae go through three instars, the first and third of which are pictured here. After dispersal from the carcass, larvae crawl into the soil to pupate for three weeks in the lab, going from the pale pupa shown, to the darker pupa nearing the completion of development. The adult emerges from the soil and is sexually mature two weeks later. Photograph by Thomas Houslay.

2.2 BURYING BEETLES AS A STUDY SYSTEM

As a laboratory organism, the burying beetle *Nicrophorus vespilloides* has many beneficial characteristics that lend itself to the study of parental care, sexual conflict, and interspecific interactions. Pukowski (1933) was among the first to study the behaviour of burying beetles, with a highly detailed account of the parental care behaviours of *N. vespillo*. Parental care likely evolved in *Nicrophorus* to safeguard the carrion breeding resource, as carrion is an ephemeral bonanza resource, which attracts high levels of inter- and intra-specific competition (Clutton-Brock, 1991; Scott, 1998b). The costs and benefits of the burying beetle's elaborate parental care has been extensively studied (Anduaga and Huerta, 2001; Eggert, Reinking and Müller, 1998; Lock, Smiseth and Moore, 2004; Meierhofer, Schwarz and Müller, 1999; Rauter and Moore, 2002; Satou, Nisimura and Numata, 2001; Scott, 1998a, 1989). Burying beetles have also become a well-utilised system for understanding sexual conflict (Boncoraglio and Kilner, 2012; Eggert and Sakaluk, 1995; Kilner et al., 2015; Pilakouta, Richardson and Smiseth, 2016) and sexual selection (Carter et al., 2015; Hopwood et al., 2016). They can also be used in experiments in the field to test the factors that influence group tolerance

and cooperation (Eggert and Müller, 1992; Eggert and Sakaluk, 2000; Sun et al., 2014).

Burying beetles are an excellent model organism in which to perform long-term evolutionary experiments. In the laboratory, *N. vespilloides* has a generation time of six weeks (Figure 2.1) making experimental evolution and artificial selection experiments feasible within a relatively short time frame. The set up in the lab is also conducive for such experiments. Individuals are kept in their own box and can therefore be given a unique ID (see below). Knowing the sire and the dam of each individual allows the construction of pedigrees where the relatedness between individuals is known. Such information is crucial for quantitative genetic studies to understand the genetic component of traits in order to predict their evolutionary potential. Unique IDs match an individual with its phenotype, which is useful for artificial selection experiments where a subset of the population is retained based on their phenotype.

Parental care in *N. vespilloides* is facultative, meaning that parents can be removed experimentally before larvae hatch and larvae will still successfully complete development (Capodeanu-Nägler et al., 2016; Eggert, Reinking and Müller, 1998; Schrader, Jarrett and Kilner, 2015b). This meant I could create a binary treatment where parents are either present in the breeding box or they have been removed before their larvae hatch. I will always refer to parents being present to care, provision, and interact with their offspring as "Full Care". These data will always be shown in red for this thesis. For the "No Care" treatment, I removed parents from the breeding box ~53 hours after pairing, when carcass preparation was complete and the clutch complete (Boncoraglio and Kilner, 2012). Data from the No Care treatment will always be in blue for this thesis.

There are large implications for larvae when parents are removed. Firstly, removing parental care results in half of broods failing in the first instance; that is, having no larvae completing development and dispersing from the carcass (Schrader, Jarrett and Kilner, 2015b; Schrader et al., 2017). Secondly, offspring rapidly adapt to a social environment without parental care (Schrader, Jarrett and Kilner, 2015b; Schrader et al., 2017). The nature of this adaptation is investigated in this thesis. Lastly, parental care changes the dynamics among offspring during development on the carcass (Schrader, Jarrett and Kilner, 2015a). Figure 2.2 shows the relationship between larval size and brood size when parents are present (Full Care, top) and when parents are absent (No Care, bottom). In the Full Care panel, there is the strong negative relationship that one expects where more siblings increase the level of competition and reduce the resources for a single larva (Mock and Parker, 1997). When parents are absent, however, the negative relationship indicative of competition still exists but only when there is more than one larva present per gram of carcass. At lower densities than this,

offspring size and brood size has a positive relationship, with a larger brood size benefitting individual offspring (Figure 2.2).

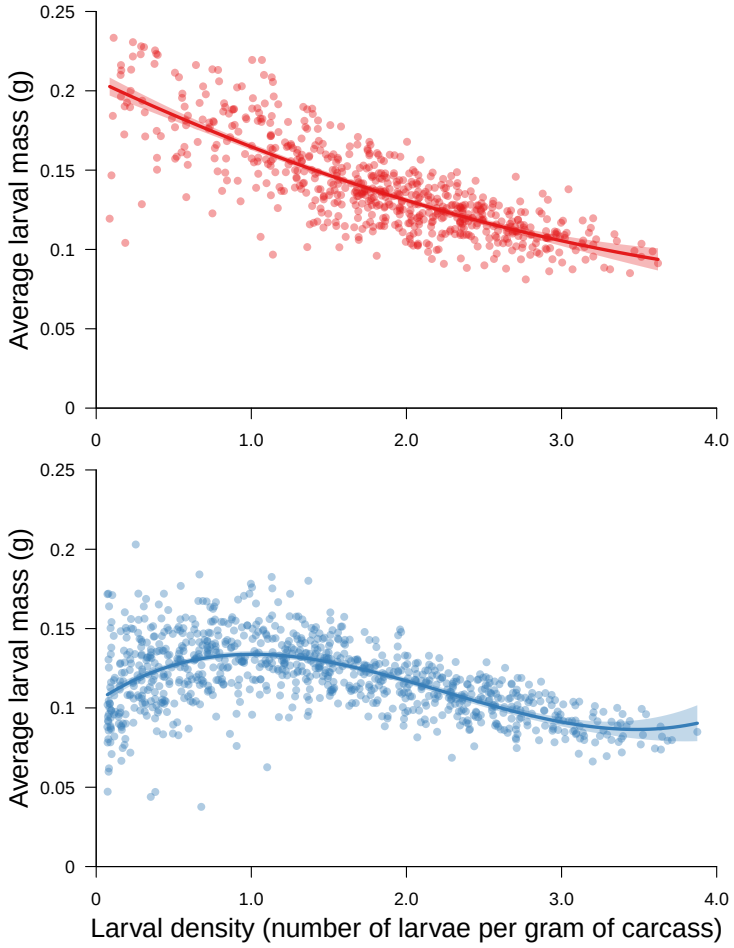


Figure 2.2 | The relationship between average larval mass and the number of larvae on the carcass depends on whether parents are present or not (Schrader, Jarrett and Kilner, 2015a). When parents are present post-hatching (Full Care, top in red), there is a strong negative relationship indicative of sibling competition for limited resources. When parents are absent (No Care, bottom in blue), this relationship is best fit by a cubic polynomial, where larval mass increases at small brood sizes, but then decreases through competition after the peak at about 1 larvae per gram of carcass. Lines shown are best fit lines from a second order polynomial for the Full Care, and a third order polynomial for the No Care, both with standard errors. Each data point is one brood from the experimental evolution populations detailed in Schrader et al. (2017).

2.3 LAB PROTOCOLS

In the laboratory, adult burying beetles are kept in their own individual boxes to track individuals and their relatedness. The generation time of *N. vespilloides*

in the laboratory is six weeks enabling experimental evolution and artificial selection experiments. We have shown already that larvae adapt to different parental environments in the burying beetle (Schrader, Jarrett and Kilner, 2015b; Schrader et al., 2017), and that parents change the outcome of sibling interactions from conflict to cooperation at low larval densities (Figure 2.2, Schrader, Jarrett and Kilner, 2015a).

2.3.1 *Feeding and maintenance*

Adult beetles were kept individually in boxes measuring 12 × 8 × 2 cm filled with compost. Each box was labelled with a unique code that had all the information required for us to know the beetle's parents, generation, sex, and population. Through these labels we maintained multiple populations of various treatments and comprehensive pedigrees for each population. Within these boxes, individuals were fed ~0.3 g of minced beef twice a week. The leftover mince from the previous feeding was often mouldy. The fungus was removed and additional compost was added at the same time as the mince. Adult beetles were kept in these boxes for two weeks until they were sexually mature.

2.3.2 *Breeding*

Once individuals were sexually mature, they were paired for breeding. A male and a female were randomly paired together, depending on the experiment. For experimental populations, breeding was random. In all cases, however, cousins and siblings did not breed together. The pair of beetles was added to a larger box measuring 17 × 12 × 6 cm half filled with fresh compost. A mouse carcass sourced from Live Foods Direct Ltd. was weighed and recorded and placed into the box, after which the pair of beetles was added.

The pair of beetles prepared the mouse carcass for the first two days, whilst the female laid in soil. At ~53 hrs, when carcass preparation and egg laying had been completed, the parents were removed in the No Care treatment. For the Full Care treatment, the parents were left in the box until the larvae dispersed, typically eight days after pairing.

2.3.3 *Dispersing*

Eight days after pairing, the larvae had completely eaten the carcass and were ready to complete development. The larvae at this stage are third instars, and can be seen crawling across the surface of the soil, in search of a suitable place to metamorphose. The contents of the breeding box were emptied into a tray to

better see and search for the larvae within. At this stage the parents are either retained or disposed, depending on the experiment, for the Full Care treatment (no parents should be present at dispersal for the No Care treatment). The larvae resulting from the breeding event were carefully collected (including hunting through the remains of the mouse) to ensure the whole brood was accounted for. The brood was counted and excess soil removed. The brood was then weighed to the nearest 0.0001 g. These data were our estimates for fitness. Average larval mass, calculated as the total brood mass divided by the number within the brood, is the most accurate representation of fitness, given the role of size in determining breeding success as an adult (Bartlett and Ashworth, 1988; House, Hunt and Moore, 2007; Otronen, 1988; Smith, 2002; Steiger, 2013).

The larvae, after they have been weighed, were placed into an eclosion box, measuring 10 × 10 × 2 cm, with 25 individual cells, each 2 × 2 × 2 cm. An individual larva was placed in each cell and covered with peat that was sifted to remove large chunks of soil. Each box holds one brood. Water was sprayed over the top to prevent desiccation during subsequent development, which typically last 18–21 days.

2.3.4 *Eclosing*

During days 17–21 post-dispersal, the eclosion boxes were checked each day. Full development is marked by the darkening of the elytra, which goes from ruddy orange to black over the course of the last few days. Individual beetles were then retained, sexed, and placed into pre-made individual boxes. Their unique identity code was assigned at this point. Three males and three females were typically retained per family, contributing to a global stock from which breeding pairs were haphazardly determined.

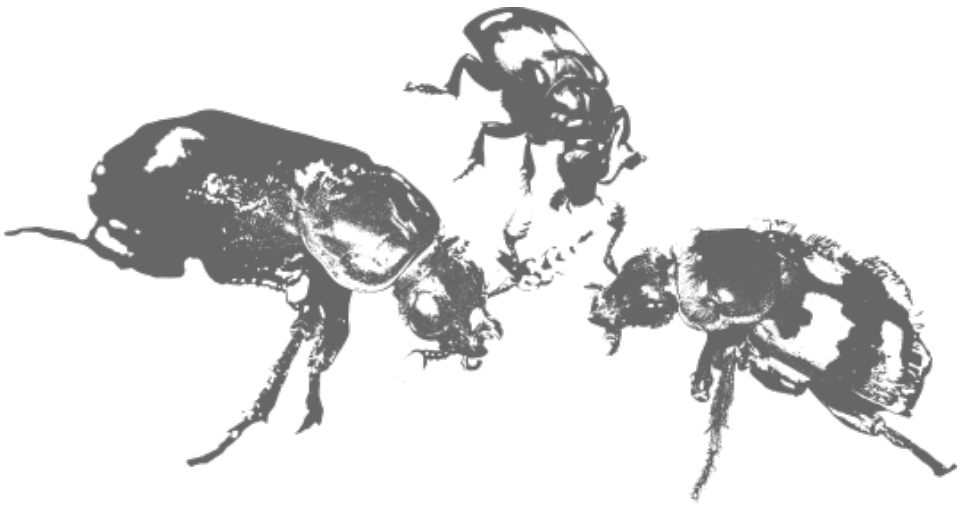
2.3.5 *Measuring pronotum width*

Adult size in an important phenotype to measure in *N. vespilloides* as it is crucial for fitness (Bartlett and Ashworth, 1988; House, Hunt and Moore, 2007; Otronen, 1988; Smith, 2002; Steiger, 2013), and also the trait that exhibits greatest variation among the member species of the genus (see Chapters 3 and 5). It was therefore important to find a quick and accurate method to measure adult pronotum width (a proxy for adult size across all beetle species, e. g. Tomkins, Kotiaho and LeBas, 2005). I developed a method where individuals were anaesthetised with CO_2 and placed flat under a Canon DSLR camera. Using a custom MATLAB script (see Appendix), I measured the number of pixels from one edge of the

pronotum to the other against a standard of known length, which in this case was the diameter of a Euro cent coin (16.25 mm).

Part II

BURYING BEETLE ECOLOGY



3

THE ADAPTIVE RADIATION OF BURYING BEETLES: COMPETITION AND CHARACTER DISPLACEMENT OF BODY SIZE

3.1 SUMMARY

Competition for resources has resulted in spectacular examples of macroevolution. These adaptive radiations are constructed through similar processes, and understanding these processes will provide a better explanation for scenarios under which adaptive diversification will occur. Here, I propose that the Silphid genus *Nicrophorus* is an example of an adaptive radiation. Burying beetles compete within and between species for carrion resources on which to breed. I use two wild populations of *N. vespilloides* that have been evolving in different guild structures, which reflect different levels of interspecific competition, to show that utilisation of the carcass niche has evolved. This most likely reflects the increased competition for carcasses from two medium-sized *Nicrophorus* species. Body size of burying beetles has been linked to carcass utilisation and provides a trait by which competition could be reduced, as smaller beetle species use small carcasses and large species use large carcasses. Using a comparative approach, I find that closely related *Nicrophorus* species are more different in body size when they are in sympatry than when they are in allopatry, indicating competition is acting on the evolution of body size. Parental care is exhibited by all *Nicrophorus* species and most likely is the key innovation that facilitated the adaptive radiation of the burying beetles through parental effects on body size.

3.2 INTRODUCTION

Adaptive radiations provide some of the most spectacular examples of macroevolution: Darwin's finches (Grant and Grant, 2006), Caribbean *Anolis* lizards (Losos et al., 1998; Losos, 2009), and East African cichlids (Seehausen, 2006) are all wonderful examples of the immense array of phenotypic variation that can arise from selection to occupy new niches. However, not all instances of adaptive radiation are as striking as these, and the criteria used to determine whether or not a lineage is an adaptive radiation have been the subject of much debate (Losos and Miles, 2002). Schluter (2000) defines an adaptive radiation as '*…the evolution of ecological and phenotypic diversity within a rapidly diverging*

lineage… [involving] the differentiation of a single ancestor into an array of species that inhabit a variety of environments and that differ in the morphological and physiological traits used to exploit those environments'. Schluter (2000) additionally puts forward four criteria on which an adaptive radiation should be judged: common ancestry; pace of speciation; phenotype–environment correlation; and trait utility. Based on evidence from the literature that I have synthesised, I propose that the Silphid genus *Nicrophorus* is an adaptive radiation as it fits all these criteria. I then test this proposition by describing an experiment on one *Nicrophorus* species and by executing a broader-scale phylogenetic analyses of the whole genus.

3.2.1 *The adaptive radiation of* Nicophorus*: evidence for Schluter's four criteria*

1. Common ancestry and 2. Rate of speciation

The burying beetles (*Nicrophorus* spp.) are a genus in the Silphidae family whose member species are found around the world, primarily in temperate regions of the Northern Hemisphere (Sikes and Venables, 2013). A recent molecular phylogeny reveals that they have a common ancestor (Sikes and Venables, 2013), a criteria Schluter uses to rule out paraphyletic convergent niche evolution. Furthermore, their speciation appears to be relatively rapid with respect to the other genera in the Nicrophorinae sub-family. *Nicrophorus* comprises 68 extant species, where the other two genera, *Ptomascopus* and *Eonecrophorus* have four and one, respectively (Sikes, Madge and Newton, 2002). Though the radiation has occurred over 113 million years, it has been a rapid process relative to its sister genera (see Losos and Miles, 2002, for thoughts about sister-clade comparisons).

3. Correlation between phenotype and environment

This criterion is key to demonstrating that any radiation is adaptive (Schluter, 2000). Burying beetles, like all the Silphids, are carrion feeders that also breed on carrion. Competition for vertebrate carcasses is high and burying beetles compete with bacteria, fungi, other insects, scavengers, as well as congenerics and conspecifics for them. Unlike all the other Silphids, *Nicrophorus* beetles have evolved elaborate parental care behaviour, where parental beetles shave and bury the carcass, cover it in anti-microbial anal exudates, and regurgitate pre-digested carrion to their offspring after they hatch (see Chapter 2). Parental care in this genus likely evolved as a way of defending, and fully utilising, the vertebrate carcass, which is a highly ephemeral resource (Clutton-Brock, 1991; Scott, 1998b).

In burying beetles, competition for carrion is likely to have driven adaptations that are associated with reproductive isolation. Interspecific competition like this is a key process in the classic examples of adaptive radiation (Schluter, 2000). For example, Darwin's finches compete for seeds (Grant and Grant, 2006), and the Caribbean anoles compete for space (Schoener, 1975). The reason is that selection favours phenotypes that reduce competition between species, partitioning the niche. As exploitation of a particular niche commonly derives from one trait, this causes divergence in this trait in order to reduce competition in a process called character displacement. Character displacement is observed between two species when they are in sympatry. For example, the anole *A. carolinensis* moved to higher perches after the introduction of the congener *A. sagrei*, showing character displacement in terms of space use. This process also set in motion the evolution of a morphological innovation to reinforce the partitioning of the ecological niche; in only 20 generations *A. carolinensis* evolved larger toepads better adapted for the new environment (Stuart et al., 2014).

What equivalent character is displaced in *Nicrophorus*, to partition the carrion niche, and how could it contribute to reproductive isolation? Ecological data qualitatively suggest that burying beetles divide up the carrion niche by specialising on differently-sized carrion. Importantly, this is determined by their relative body size (Scott, 1998b). Populations that are locally-adapted to different carrion sizes may have evolved different strategies to do so. Populations of larger beetles could have evolved reduced clutch size in order to distribute more resources to fewer offspring, or evolved greater reliance on parental provisioning. Hybrids could potentially suffer reduced fitness through social epistasis or a mismatch of genes for differing strategies, which ultimately disrupts optimal body size (see Chapter 7). Scott (1998b) described how burying beetle guilds are structured through body size, with a large species (*N. americanus* in North America and *N. germanicus* in Europe) and several medium and small species making up the guilds on these two continents. In this way, guilds of burying beetles can exist in sympatry by sub-dividing the carrion niche. Field and laboratory studies also indicate that larger species are better able to utilise larger carrion as a breeding resource, whereas smaller species tend to utilise smaller carrion (Scott, 1998b; Trumbo, 1990, 1992; Wilson, Knollenberg and Fudge, 1984). Thus there is a clear link between beetle body size (phenotype) and the resource it uses for reproduction (environment). However, the correlation between phenotype and the environment has not yet been rigorously tested in a phylogenetic context.

4. Trait utility

The next key issue is to determine whether the fit between phenotype and the environment enhances fitness in the matched environment. This criterion is ful-

filled for *Nicrophorus* species because beetle body size is not only correlated with a key environmental resource, but also affects reproductive success on that size of carrion. Within *N. vespilloides*, Hopwood et al. (2015) showed that the size of the individuals influenced how effectively they could breed on carrion of different size, with smaller individuals better able to utilise smaller carrion, and larger individuals better able to utilise larger carrion, which would involve concealing the carcass through burial as well as the production of offspring. Furthermore, in *N. vespilloides*, the size of the carrion resource positively influences the size of the offspring reared upon it (Andrews, Kruuk and Smiseth, 2016; Smiseth et al., 2014, but see Chapter 4). This, in effect, creates a feedback loop between body size and carrion size that can accelerate differences in carrion use to further minimise competition from other burying beetle species. Furthermore, parental care plays an important role in creating this feedback loop because parents determine the resources available for each of their developing offspring and therefore the size they can attain as adults (Andrews, Kruuk and Smiseth, 2016; Smiseth et al., 2014).

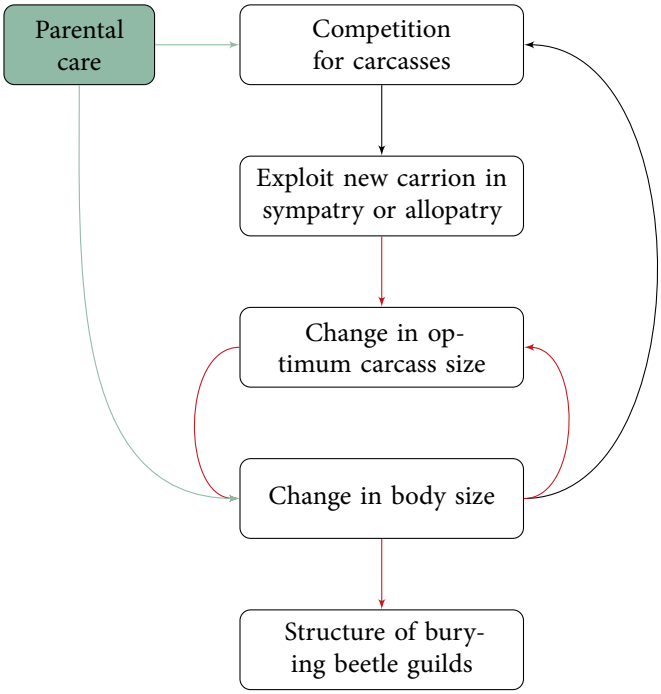


Figure 3.1 | A hypothesised mechanism for the adaptive radiation of the burying beetles, via changes in beetle body size. The red arrows indicate the steps in logic I test in this chapter, using experimental and comparative approaches. The blue arrows indicate the role of parental care in this process, and will be discussed later.

3.2.2 *The adaptive radiation of the burying beetles*

The evidence from the literature thus strongly suggests that burying beetles have adaptively radiated under competition for carrion to breed upon. In Figure 3.1, I outline how such a process might have happened. Here, burying beetles are imagined to compete for vertebrate carcasses, which vary in size. Competition for carcasses leads to selection for using a novel carrion resource that differs in size from the carrion used currently. This event could occur in sympatry or in allopatry through dispersal to a new patch with a novel carrion resource. Selection then acts to optimise fitness on this new carrion resource by evolving adult body size, and as a result causing the structuring of burying beetle guilds.

In this chapter, I used two complementary approaches to test the hypotheses outlined in Figure 3.1. The first uses a natural experimental system comprising two populations of a small burying beetle species, *N. vespilloides*. The two populations are part of two different guilds of burying beetles that differ in their structure. One population comprises two species of burying beetles, while the other comprises four species. Therefore the intensity of interspecific competition for carrion within each population differs. Putting this information into the logic outlined in Figure 3.1, it would follow that the two populations of *N. vespilloides* have different opportunities to exploit the carrion niche. With less competition for carcasses from congeners, *N. vespilloides* is therefore predicted to utilise larger carrion, which can yield more, larger offspring (Smiseth et al., 2014). Larger individuals better utilise larger carcasses to convert it into more and larger offspring (Hopwood et al., 2015). As in Figure 3.1, this change in body size would feedback into the optimum carcass size, resulting in larger beetles that better utilise larger carcasses, and ultimately structure burying beetle guilds. If competition for carrion results in the structuring of burying beetles, I predict that the population with less competition would consist of larger bodied individuals and have a larger optimum carcass size than the population with more competition.

My second approach uses a database of 49 burying beetle species that were part of a recently published molecular phylogeny (Sikes and Venables, 2013). Also included in Sikes and Venables (2013), were the locations of the trapped individuals used in the study, which I used to calculate the geographic ranges of each species. Body size data for each species were collected from current museum specimens, so I could ask whether competition between burying beetles (i. e. their co-occurrence) leads to divergence of body size on a global scale. If competition within the carcass niche has led to the radiation of the *Nicrophorus* genus, burying beetle species that are sympatric are predicted to be more different in body size than species that are allopatric.

Finally, I conclude by using the phylogeny and body size data to test if the evolution of parental care could be the key contribution to the adaptive radiation of the burying beetles through its effect on body size (Figure 3.1), and its role in ameliorating competition for the carcass more directly, by concealing the carrion resource from rivals through burial.

3.3 METHODS

3.3.1 *Local dataset*

Gamlingay and Waresley Woods

Experiments were conducted on *N. vespilloides* individuals collected from two sites in Cambridgeshire, Waresley Woods (W) and Gamlingay Woods (G). The two sites are geographically close (2.5 km apart, Pascoal and Kilner, 2017), but exhibit different burying beetle guild structures. Gamlingay has four species of *Nicrophorus—N. vespilloides*, *N. investigator*, *N. interruptus*, and *N. humator* (see Figure 3.2)—and Waresley only has two—*N. vespilloides*, and *N. humator*. Despite being so close, mark-recapture did not detect any migrants between the sites, presumably because of the farmland between (S.-J. Sun, unpublished data). However, population genetic studies using neutral markers suggest Gamlingay and Waresley populations of *N. vespilloides* are not genetically distinct (though other populations in Cambridgeshire are genetically distinct, Pascoal and Kilner, 2017).

Nevertheless, the guild structure is consistent across multiple years, and significantly different between sites (S.-J. Sun, unpublished data). The two additional species in Gamlingay have intermediate body sizes to the small *N. vespilloides* and the large *N. humator* (Table 3.1). I predicted that the presence of interspecific competition from *N. investigator* and *N. interruptus* would have evolutionary consequences for the Gamlingay *N. vespilloides* populations. Specifically, I predicted that *N .vespilloides* adults from Gamlingay Woods would be smaller than those from Waresley Woods and would specialise on smaller carrion as a consequence of the greater levels of interspecific competition they experience.

To test for these predicted differences in body size, I collected wild *N. vespilloides* from both sites using a Japanese beetle trap and measured the pronotum width of all individual beetles. To determine whether any differences in size that I might detect were genetic, or due to environmental effects, I brought wild-caught beetles from the two populations back to the lab and bred them in a common garden environment, by giving beetles from the two populations carcasses from the same narrow range in carcass mass (8–14 g). I then measured the difference in size of the offspring, when they reached adulthood, by photographing them in

a standardised manner (see Chapter 2). A custom MATLAB script was then used to measure the pronotum width from the digital images obtained (see Chapter 2).

Next, I tested the prediction that *N. vespilloides* from Gamlingay and Waresley is locally adapted to use a different portion of the carrion niche. In the laboratory, F_1 descendants of wild-caught beetles from each population were provided with three categories of carrion size on which to breed, to simulate the range of carcass sizes these beetles are likely to find in the wild. These were small (8–12 g), medium (25–30 g), and large (40–50 g). Beetles within each population were randomly allocated to each treatment.

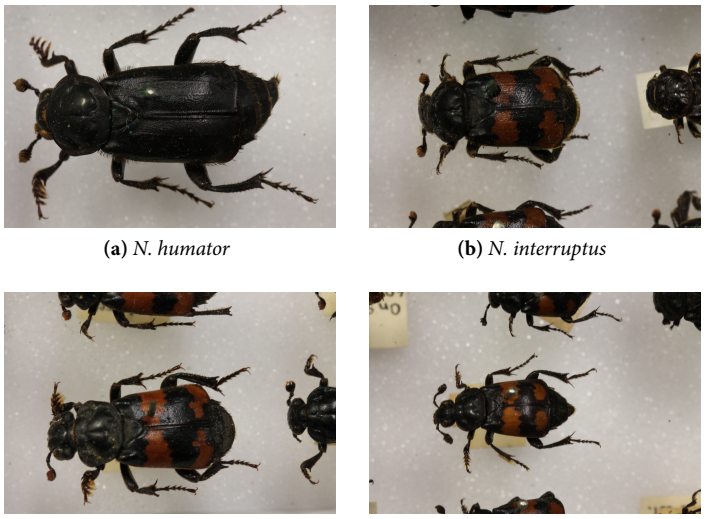
Statistical analysis

I analysed body size data from 1086 field-caught individuals across two years (2014 and 2015), using a linear model to test for a difference in pronotum width between Gamlingay and Waresley Woods, controlling for the sex of the beetle and the year in which they were collected. I also analysed the body size data for the F_1 generation of both populations in the lab controlling for the size of the carcass, the size of the parents, the number of siblings each individual had, and the family ID as a random term, using the `R` package `lme4` (Bates et al., 2015).

The carcass experiment was analysed using models with the interaction term of carcass treatment and population, with the size of each parents added as covariates. For average larval mass, the number of larvae was also added as a covariate (see Schrader, Jarrett and Kilner, 2015a). Independent of the results of the global model, I planned post-hoc contrasts between populations for each carcass treatment where I directly compared how each population performed in each carcass treatment separately. Bonferroni corrections were used for the contrasts. Brood size was analysed with a generalised linear model with a quasipoisson error distribution and log link function.

3.3.2 *Global comparative dataset*

I compiled a global dataset of the burying beetles by including only the species included both in the recently published *Nicrophorus* molecular phylogeny (Sikes and Venables, 2013) and the complete classification of the genus (Sikes, Madge and Newton, 2002, but see Sikes and Mousseau, 2013; Sikes, Trumbo and Peck, 2016). I calculated the pace of speciation in the *Nicrophorus* genus by comparing the number of species with those in the sister genera, *Ptomascopus* and *Eonecrophorus* using the method outlined in Nee, Barraclough and Harvey (1996).



(a) *N. humator* **(b)** *N. interruptus*

(c) *N. investigator* **(d)** *N. vespilloides*

Figure 3.2 | Examples of photographs from the Natural History Museum collections. All species on the recent molecular phylogeny (Sikes and Venables, 2013, Figure 3.8) were photographed. The four species are those found at one of the sites in Cambridgeshire, UK, and are commonly found across Europe.

Body size

Body size data were collected from the Coleopteran collection at the Natural History Museum in London. I photographed all of the *Nicrophorus* specimens in the collection using a mounted DSLR camera, standardised the distance between lens and specimen, and ensured the pronotum was perpendicular to the photography angle (Figure 3.2, Chapter 2). The scale was photographed anew every time the camera was moved. The scale bar thus remained constant in photographs in the same set. I used the same custom MATLAB script to measure the pronotum width of each individual (see Chapter 2 for more details). The body size data for each species used in the analysis are in Table 3.1.

Geographic range

The ranges of the *Nicrophorus* species (Figure 3.3) were extracted from the georeferenced trapping locations given in Sikes and Venables (2013). The coordinates for all species were used to create a minimum convex polygon in ARCGIS (ESRI, 2011). To ensure ranges were limited to land, I restricted the polygons to coastlines by fitting coastline polygons. The range for each *Nicrophorus* species was thus defined by a polygon of the outer georeferenced points. For Holarctic species, which span both the continents of North America and Europe, this was split into two separate polygons.

Table 3.1 | All of the *Nicrophorus* species on the molecular phylogeny for which data was collected. The sample size (N) is the number of individuals measured in the Natural History Museum collections.

Species	N	Mean pronotum width (mm)	Standard deviation
N. americanus	27	10.58	1.04
N. antennatus	8	5.92	0.76
N. apo	2	5.20	0.55
N. argutor	5	6.67	0.42
N. carolinus	40	6.99	0.93
N. charon	9	5.52	0.65
N. concolor	37	10.78	0.99
N. dauricus	6	6.77	0.52
N. defodiens	60	5.43	0.59
N. didymus	28	5.51	0.60
N. distinctus	14	6.73	0.46
N. encaustus	5	5.67	0.50
N. germanicus	24	9.92	1.38
N. guttula	50	5.72	0.73
N. heurni	12	5.35	0.55
N. humator	33	7.16	0.85
N. hybridus	8	7.26	0.89
N. insularis	8	5.87	0.48
N. interruptus	35	5.81	0.60
N. investigator	105	5.99	0.77
N. japonicus	16	6.58	0.98
N. kieticus	29	4.34	0.53
N. lunatus	5	6.65	1.10
N. maculifrons	12	5.72	0.91
N. marginatus	69	6.38	0.96
N. mexicanus	20	5.84	0.76
N. montivagus	18	4.32	0.53
N. morio	7	9.00	0.88
N. nepalensis	90	5.22	0.58
N. nigricornis	4	6.34	1.00
N. nigrita	15	6.07	0.99
N. oberthuri	9	5.51	0.60
N. obscurus	36	6.97	0.99
N. olidus	20	4.66	0.62
N. orbicollis	39	6.65	0.82
N. podagricus	80	6.10	0.62
N. przewalskii	4	6.59	0.20
N. pustulatus	18	7.05	0.93
N. quadrimaculatus	4	4.92	0.76
N. quadripunctatus	56	5.05	0.67
N. sayi	40	6.12	0.65
N. scrutator	7	5.94	1.50
N. semenowi	3	5.18	0.90
N. sepultor	12	6.12	0.54
N. smefarka	4	4.13	0.42
N. tenuipes	20	5.63	0.39
N. tomentosus	50	5.46	0.59
N. vespillo	50	5.72	0.77
N. vespilloides	70	4.83	0.59
P. morio	23	4.16	0.56

I compared each *Nicrophorus* species with all other species in the dataset in order to assess whether two species have geographic ranges that overlap, and the extent of this overlap (i. e. do both species overlap entirely, or do their range only

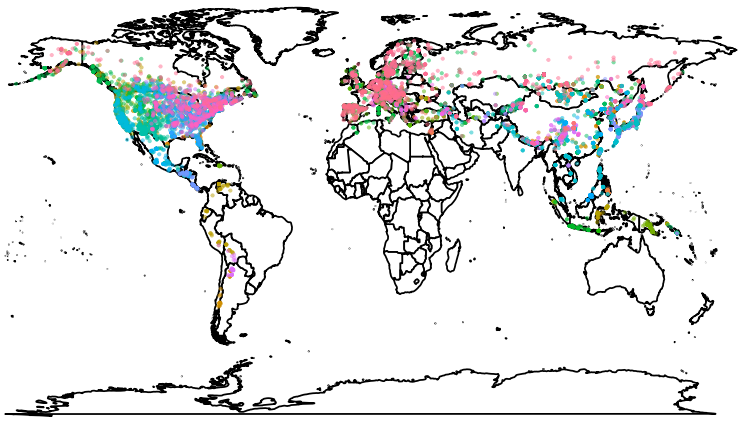


Figure 3.3 | The distribution map of all of the burying beetle species in the phylogeny. Each dot represents a record for each species as recorded from Sikes and Venables, 2013, and each colour represents a different species. The majority of burying beetle species are restricted to the northern hemisphere, but can be found along mountain ranges south of the equator.

overlap slightly). I calculated the overlap for each pair of species by calculating the geographic area each species overlapped, and dividing it by the total area of both species' ranges, that excluded the area both species shared. This method provides a symmetrical measure of the extent of overlap, and does not take into account changes in habitat, or other more fine details that could influence the ecological interactions between both species. I therefore used the extent of geographic overlap to create a two-level factor, where any overlap greater than 20% was coded as sympatric and less than 20% as allopatric (Tobias et al., 2014). I shifted this threshold at 5% increments from 5% to 50% to test how robust this assumption was, as well as testing the robustness of the results.

Mode of parental care

I classified post-hatching parental care as 'facultative', where care is not necessary for some offspring to complete development, or 'obligate', where parental care is required for all larvae to complete development, using data from the published literature and when unavailable from published literature, from personal communication with other burying beetle researchers (N = 14 species, Table 3.2). I searched Web of Science and Google Scholar for information about parental care using the species name and 'parental care', in conjunction with either 'facultative' or 'obligate' as search terms. I tested whether data from personal communications biased the analyses by running the analyses with and without these data included.

Table 3.2 | Variation in the provision of parental care across burying beetle species. 'Obligate' care means that larvae cannot survive to the third instar unless they are cared for by their parents; 'facultative' care means larvae can survive without their parents.

Species	Parental care	Source of information
N. americanus	Obligate	D. Howard, pers. comm.
N. defodiens	Facultative	Trumbo, 1992
N. humator	Obligate	BP Springett cited in Trumbo, 1992
N. investigator	Obligate	BP Springett cited in Trumbo, 1992
N. marginatus	Obligate	D. Howard, pers. comm.
N. mexicanus	Facultative	Anduaga and Huerta, 2001
N. nepalensis	Facultative	S.-J. Sun, pers. comm.
N. orbicollis	Obligate	Capodeanu-Nägler et al., 2016; Trumbo, 1992
N. pustulatus	Facultative	Capodeanu-Nägler et al., 2016; Trumbo, 1992
N. quadripunctatus	Facultative	Satou, Nisimura and Numata, 2001
N. sayi	Obligate	Trumbo, 1992
N. tomentosus	Facultative	Trumbo, 1992
N. vespillo	Facultative	Pukowski, 1933
N. vespilloides	Facultative	Capodeanu-Nägler et al., 2016; Eggert, Reinking and Müller, 1998; Schrader, Jarrett and Kilner, 2015b

Statistical analysis

To test whether there was a relationship between the mean difference in body size between two species and the extent to which both species overlap, whilst controlling for phylogeny, I used a phylogenetic Mantel test (Harmon and Glor, 2010), using the two-level factor of 'sympatric' or 'allopatric'. Due to the low power of the Mantel test, I found no pattern between range overlap and body size differences ($r = 0.064, P = 0.12$). I subsequently followed Tobias et al. (2014) and selected the closest related species in sympatry and the closest related species in allopatry. With this method, I analysed each comparison between each *Nicrophorus* species as a data point, where, for example, the first 48 rows are the interactions *N. americanus* (the first species alphabetically) has with each of the other species, including itself which is then excluded. I then used a linear mixed model (LMM, Bates et al., 2015) to regress the absolute difference in body size between the two species against the two-level sympatry/allopatry factor, keeping species one and species two as random terms in the model. If two or more species were close on the phylogenetic tree to the focal species (i. e. two sister species), one of these species was chosen at random (Tobias et al., 2014).

I mapped body size onto the dated, molecular phylogeny, which provides the basis for the analysis of the global dataset (Figure 3.8). I also mapped the mode of parental care (obligate or facultative) onto the phylogeny, excluding species

without data. I used a phylogenetic least squares regression (PGLS, Orme et al., 2013) to analyse the relationship between body size and mode of parental care.

3.4 RESULTS

3.4.1 *Local dataset*

Body size

Wild-caught individuals from Gamlingay and Waresley Woods did not differ in body size between the two populations ($t = -0.50, P = 0.62$). Males and females within populations were also of a similar size ($t = 1.23, P = 0.22$). Individuals caught in 2015, however, were smaller than those caught in 2014 ($t = -2.73, P = 0.01$). The lack of difference in body size between populations persisted in laboratory bred F_1 beetles ($\chi^2_1 = 0.28, P = 0.59$).

Carcass mass

In general, I found large effects of carcass size on offspring size for both populations. Smaller carcasses yielded smaller offspring ($F = 24.23, P < 0.001$). I also found a main effect of population of origin on offspring size: Gamlingay beetles produce larger offspring, irrespective of carcass size ($F = 8.81, P = 0.004$). To understand the source of this difference between populations, I compared them separately within the three carcass treatments. Post-hoc comparisons of the carcass size treatments revealed the effect of the population on offspring size was driven mainly by a different response of the two populations to the medium carcass treatment: Gamlingay parents produced larger offspring that Waresley parents ($z = -3.08, P = 0.03$, Figure 3.4). I found no difference among populations for average larval mass for the other two carcass size treatments (small: $z = -0.79, P = 0.97$; large: $z = -1.24, P = 0.81$). Equally, there was no interaction between population and carcass treatment on brood size ($F = 2.57, P = 0.08$); nor were there any differences in brood size when comparing between populations in carcass treatments (large: $z = -2.08, P = 0.29$; medium: $z = -1.64, P = 0.56$; small: $z = 0.72, P = 0.98$).

I found that the relationship between larval size and number of larvae differed between carcass sizes and populations. There was a strong negative relationship between brood size and larval size on the small carcasses ($t = 7.50, P < 0.001$, Figure 3.5a), but not on either the medium or large carcasses (medium: $t = -1.16, P = 0.25$; large: $t = 1.66, P = 0.12$, Figure 3.5b and c). I did find, however, that on the medium carcasses heavier larvae were produced by Gamlingay par-

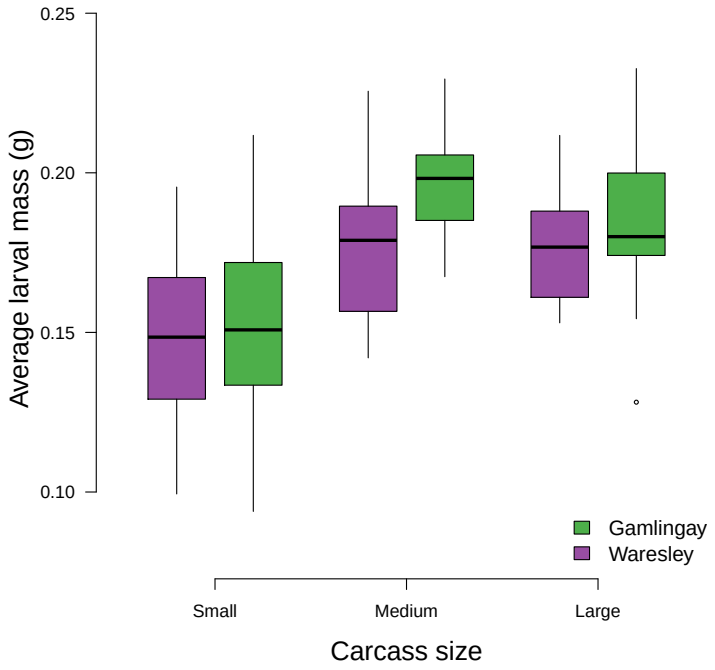


Figure 3.4 | The relationship between carrion size utility and average larval mass is different between Gamlingay and Waresley populations. On medium carcasses, Gamlingay parents produce larger offspring, but not on small or large carcasses.

ents independent of brood size ($t = -3.47$, $P = 0.001$). No such effect was found for the small ($t = -0.34$, $P = 0.74$) or large carcasses ($t = -0.38$, $P = 0.71$).

I found a significant interaction between the mean size of the parents and the size of the offspring they reared on a medium carcass ($t = -2.36$, $P = 0.024$, Figure 3.6). Large Gamlingay parents produced larger offspring, whereas large Warseley parents produced smaller offspring.

3.4.2 *Global dataset*

Body size in sympatry and allopatry

The variance of absolute body size differed between allopatric and sympatric species ($F_{1,84} = 4.14$, $P = 0.045$, Figure 3.7). To standardise the variance, I took the natural logarithm of the body size difference between the focal species and the most closely related species in sympatry and the most closely related species in sympatry. The difference in body size between two species was greater when both species overlapped and were in sympatry ($\chi^2_1 = 6.42$, $P = 0.011$, Figure 3.7). Shifting the threshold for sympatry from 20% upwards did not change this

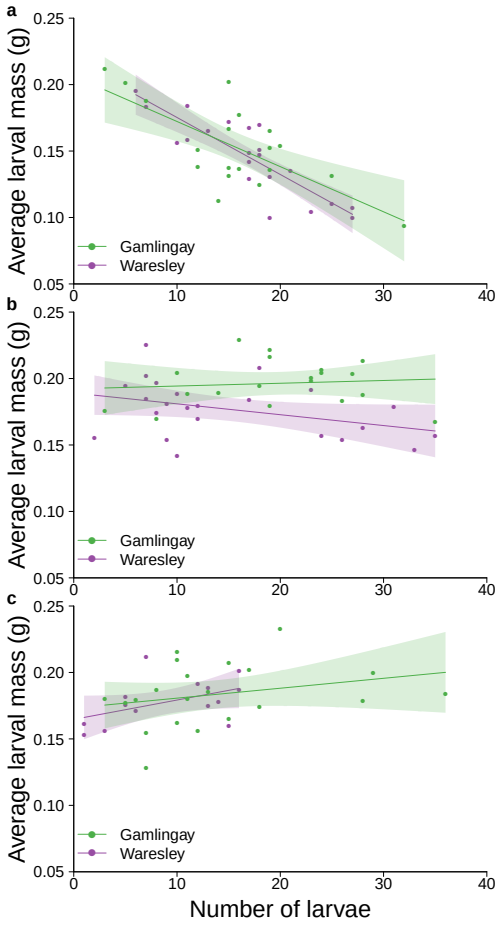


Figure 3.5 | The trade-off between larval size and number of larvae depends on the carcass on which the larvae developed and the population of the parents. On a small carcass (**a**) there was a strong negative relationship between larval size and the number of larvae in the brood. On a medium carcass (**b**) there was a significant interactions between larval size and larval number for the two populations, with Gamlingay parents raised offspring that were significantly larger independent of brood size. On a large carcass (**c**), there was no relationship between brood size and larval mass, nor an effect of the population.

pattern (all $P < 0.047$). Only when sympatric species were taken to overlap by 10% or less did this pattern break down (all $P > 0.510$). Taking those same species pairs, I also found allopatric pairs were significantly closer on the phylogenetic tree ($\chi^2_1 = 34.00, P < 0.001$). In essence, species are found in sympatry with their most closely related species, but those that are sympatric showed more divergence in body size.

Mode of parental care across the Nicrophorus *phylogeny*

I found body size has a phylogenetic signal (PGLS regression, $\lambda = 1.01$, 95% CIs = 0.94–1.02, Figure 3.8). Species more closely related to each other have similar

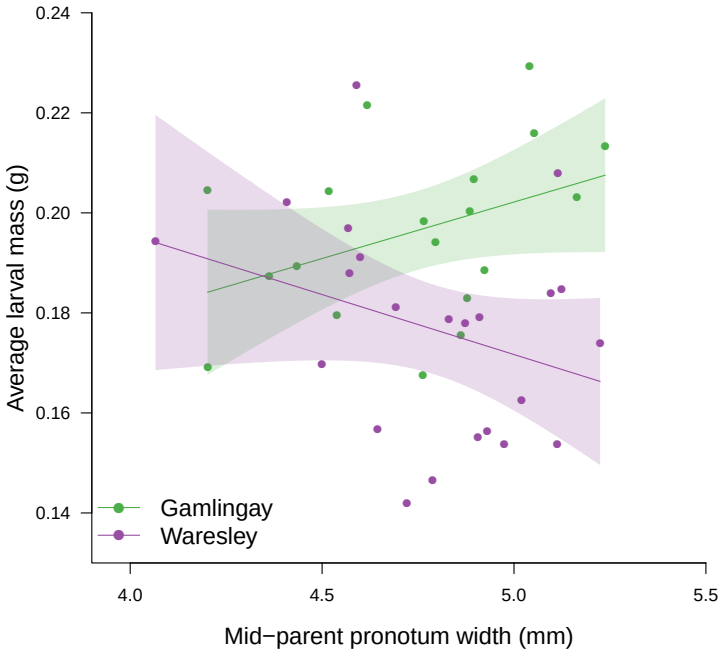


Figure 3.6 | Adult size influences the ability to produce large offspring, and this differs between Gamlingay and Warseley populations, but only on a medium carcass. Gamlingay parents are more successful at producing larger larvae on a medium carcass.

body sizes. The mode of parental care was highly correlated with body size across the genus; larger species had obligate parental care (est ± s.e. = 1.57 ± 0.66, t_{12} = 2.50, P = 0.035). Systematic removal of data obtained through personal communication (Table 3.2) did not affect the results. I removed *N. americanus* (est = 0.88 ± 0.35, t_{11} = 2.54, P = 0.028), *N. marginatus* (est = 1.72 ± 0.72, t_{11} = 2.40, P = 0.035), and *N. nepalensis* (est = 1.52 ± 0.71, t_{11} = 2.13, P = 0.056) from this analysis separately, and performed the analysis without all three species (est = 0.85 ± 0.42, t_9 = 2.05, P = 0.07). The results excluding *N. nepalensis*, and excluding all three species, were still marginally significant. More importantly, a large effect size in the same direction was retained; that is, larger species have obligate care.

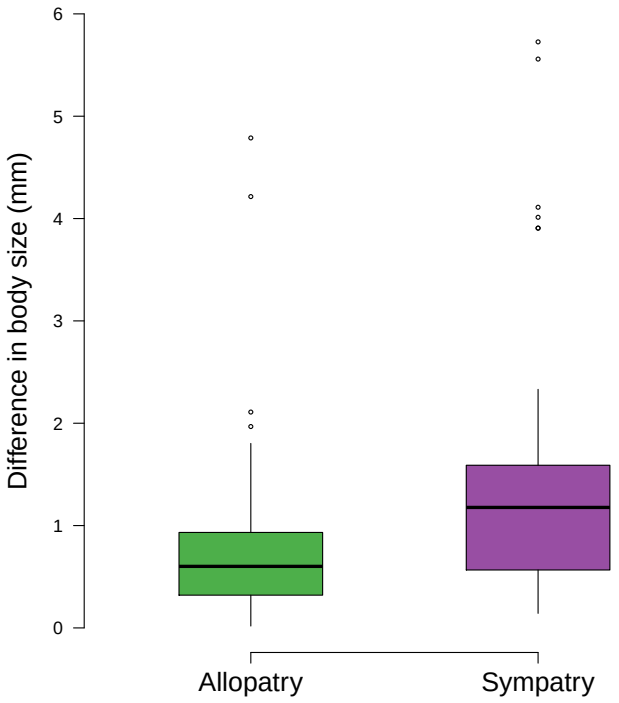


Figure 3.7 | The body size differences between the most closest related species that are either sympatric or allopatric. Species pairs in sympatry have a greater difference in body size than species pairs in allopatry.

3.5 DISCUSSION

3.5.1 *Does competition influence carrion use and body size: a comparison of* N. vespilloides *in Gamlingay and Waresley Woods?*

Contrary to my prediction, I found no evidence that the body size of wild-caught individuals differed between Gamlingay and Waresley Woods. Nor, in general, could I detect a difference in body size in the F_1 generation, after breeding individuals in a common environment in the lab. However, I did find that individuals from the two different populations used carrion differently, but only when given medium (20–25 g) carcasses to breed upon. On these carcasses, Gamlingay beetles tended to produce smaller broods with significantly larger offspring compared with Waresley beetles. However, since wild-caught adults did not differ in body size, presumably Gamlingay beetles seldom breed on 20–25 g carrion in nature, despite the small mammal compositions not differing (S.-J. Sun, unpublished data). Perhaps this size of carcass is used instead by their larger rivals, *N. interruptus* and *N. investigator* (neither of these species occurs

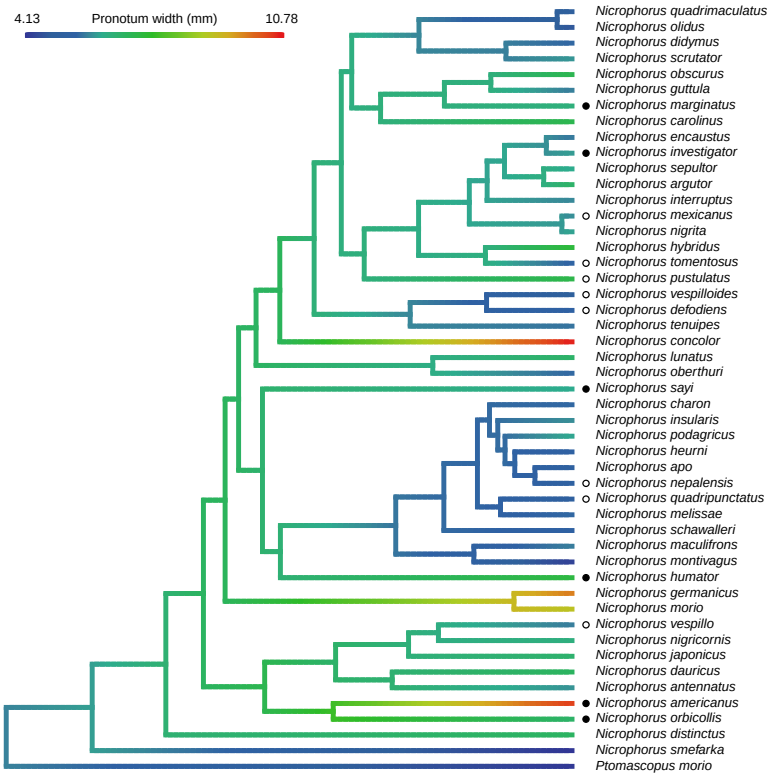


Figure 3.8 | Body size of every *Nicrophorus* species in the molecular phylogeny (Sikes and Venables, 2013). White circles indicate species that have facultative post-hatching parental care, whereas the black circles are species with obligate care. Species without data on the mode of care have no circles.

commonly in Waresley Woods). In short, my experiments provide indirect evidence that competition for carrion among burying beetles could indeed partition the carrion niche by causing beetles to specialise on smaller (or larger) carrion. However, unlike previous work (e. g. Andrews, Kruuk and Smiseth, 2016) my results suggest that a change in body size does not automatically then follow from a change in the carrion size used for reproduction.

Selection therefore appears to act on Gamlingay parents to produce larger offspring on the size of carrion where competition between the larger species is greater. The medium carcasses do not limit the size of offspring through the strong negative relationship between larval size and larval number that is present on smaller carcasses (Figure 3.5), and so offers the only environment where parents can achieve the offspring phenotype selection favours. In a highly competitive environment, large beetles have greater chance of securing carcasses on which to breed, by fighting off hetero- and conspecifics (Otronen, 1988). Such a pattern does not exist on large carcasses as these are unlikely to be used as

a breeding resource by *N. vespilloides*, as they are too large to be successfully concealed by such a small species.

One unexpected difference between Gamlingay and Waresley was that the size of the parents influenced the size of offspring reared on a medium sized carcass. Large parents from Gamlingay produced larger offspring, whereas Waresley parents of the same size produced smaller offspring (Figure 3.6). Again this result suggests that parents in the two populations are adapted to use differently sized carcasses. Perhaps Gamlingay parents are adapted to lay smaller clutches in general because they usually breed on smaller carrion. On a large carcass this means they produce larger offspring (Schrader, Jarrett and Kilner, 2015a). This result reinforces the results from Hopwood et al. (2015), who found larger individuals performed better on larger carrion. This feedback can reinforce niche partitioning by *Nicrophorus*, by ensuring that sympatric species remain specialised on carrion of a particular size. Future work should examine the carcass niche between the small, medium and large carcass treatments. A continuous range of carcasses would allow greater resolution to determine at what point the optimum carcass mass between population changes. The carcass niche utilised by each species differs (Scott, 1998b; Trumbo, 1990, 1992), but our knowledge of how the carcass niche is actually partitioned between populations of the same species, but in different guild structures, is as yet unknown. Of course, this data stems from two burying beetle populations. Similar data from populations that have a range of guild structures would help solidify and generalise these conclusions.

3.5.2 *Does competition among burying beetles cause divergence in body size: interspecific comparisons with a global dataset*

The first analysis—the Mantel test using the whole dataset with all species interactions—found that differences in body size between two species were broadly similar whether pairs of species were in allopatry or sympatry. There might be a biological explanation for this finding. Geographic ranges are notorious for shifting more easily and rapidly when environments change (Davis and Shaw, 2001; Parmesan et al., 1999). Therefore, inferring methods of speciation from such data can be extremely difficult (Barraclough and Vogler, 2000). In addition to a continual shifting of geographic range, random or not, evolution keeps ploughing on after species diverge. This could lead to greater differences between species, which may possibly be independent of the trait by which they may have first diverged (Letten and Cornwell, 2015). Younger clades could therefore be strongly selected for different ecological characteristics to further reduce competition (Pearse, Jones and Purvis, 2013). This is likely to also be the case within the *Nicrophorus* as habitat is strongly divergent between the species where

such data is abundant (Scott, 1998b), which thus reduces the effectiveness of large-scale biogeographical data.

The second analysis focused only on the most closely related species in sympatry and allopatry. I found that sympatric species were more different in terms of body size than their closely related species in allopatry (Figure 3.7), which is what one would expect if body size aided the partitioning of the carrion niche. Body size in *Nicrophorus* evolves in a Brownian manner, but it is the selective assortment of communities of burying beetles that structures guilds and enables species to exist in sympatry. What is more, I found that the variance in body size differences was greater in the sympatric species. Greater variance in body size of sympatric species indicates phenotypic overdispersion in burying beetle communities, a hallmark that body size is the trait by which competition between species is reduced (Cavender-Bares et al., 2004).

In addition, I found that species living in allopatry were also most likely to have recently diverged whereas species in sympatry were also phylogenetically further away from the focal species. This is consistent with the suggestion that beetles move to exploit a new carrion resource and, in so doing, become reproductively isolated in allopatry. It also suggests that partitioning of the carrion niche through changes in body size happens only on secondary contact. This is the classical model by which most adaptive radiations have been hypothesised to proceed (Schluter, 2000; Stroud and Losos, 2016). It is perhaps best exemplified by archipelago radiations like Darwin's finches (Grant and Grant, 2008), where a population disperses to a new island and diverges in allopatry through adaptation to the new environment or genetic drift. The two populations (now species) then contact one another again and start competing. Divergence in a trait reduces this competition resulting in character displacement of the trait. In the burying beetles, however, it appears as if there is lineage sorting (Tobias et al., 2014), whereby the diversity in body sizes that may have evolved in allopatry sorts into guilds to minimise competition. Perhaps it is this process of phylogenetic overdispersion (Cavender-Bares et al., 2004) that allows species that vary in size to persist.

In a final set of analyses, I tested for evidence that the evolution of parental care is the key innovation that contributed to the radiation of the burying beetles, through its association with body size. I found that parental care and body size are associated with each other: larger species depend on parental care, whereas smaller species do not require care to survive (though it improves survival rates, Eggert, Reinking and Müller, 1998). I argue that this is consistent with parental care being a key innovation that facilitated the diversification of the *Nicrophorus* genus.

Key innovations are traits that allow a lineage to interact with the environment in a new way, increasing their ecological opportunity (Stroud and Losos, 2016; Yoder et al., 2010), and most likely promote diversification (Galis, 2001; Hunter, 1998; Rabosky, 2014). For example, the hypocone dentition of rodents facilitated rodents in expanding into greater herbivorous niches (Hunter and Jernvall, 1995), and the toepad of *Anolis* lizards is a morphological trait, the evolution of which has enabled adaptation to new perch heights (Larson and Losos, 1996; Stuart et al., 2014)

In a similar vein, I suggest that parental care in *Nicrophorus* accelerates diversification of body size in response to the ecological opportunity of using new sizes of carrion to breed upon (Donoghue, 2009; Marazzi et al., 2012; Werner et al., 2014). Parental care acts directly to reduce competition over carrion because it helps conceal carrion from rivals. Parents roll and shave and bury carcasses to defend this valuable resource from takeovers by rivals (Scott, 1998b). More significantly for its role as a key innovation, parental care also influences body size within species. Brood size is a key determinant of offspring size (Schrader, Jarrett and Kilner, 2015a), determined partly by clutch size and partly by parents who partially cannibalise their brood (Bartlett, 1987). In addition, parents regurgitate pre-digested carrion to offspring which increases the size their larvae attain by the time they disperse from the carcass (Eggert, Reinking and Müller, 1998).

In this chapter I have compiled evidence from the literature in concert with my own experiments and analyses, to bolster the claim that *Nicrophorus* is an example of adaptive radiation (Schluter, 2000). I have presented evidence consistent with the following conclusions: 1) Competition within *Nicrophorus* species causes a change in carrion use and, 2) might underlie population divergence and allopatric speciation. When species come into secondary contact in sympatry 3) their body size is displaced. 4) However, changes in body size do not automatically follow from a switch in carcass use but instead 5) depend, in diverse ways, on how parents divide resources on the carcass among their offspring. Thus, although competition for carrion can explain why the *Nicrophorus* genus has diversified, understanding how this happened depends on understanding the contribution of parents to diversification in body size.

4

THE EFFECT OF PARENTAL CARE AND CARCASS SIZE ON GENETIC AND PHENOTYPIC VARIATION IN THE BURYING BEETLE

4.1 SUMMARY

Natural selection acts on phenotypic variation. Determining what contributes to phenotypic variation is therefore crucial to understand how evolution can proceed. Genetic variation is one such source, but it does not always influence the phenotype. This cryptic genetic variation can have profound evolutionary implications so it is important to identify factors that contribute to the build up and expression of this hidden variation. I use a quantitative genetic experiment on the burying beetle, *Nicrophorus vespilloides*, to ask if parental care can reveal evolutionarily-relevant cryptic genetic variation. Specifically, I ask whether there are changes in additive genetic variation across two social environments (Full Care and No Care) in combination with two resource environments (Small Carcass and Large Carcass). I measured two morphological traits (larval mass and adult pronotum width) and one life history trait (development time) that have been implicated in the diversification of the *Nicrophorus* genus. I found no additive genetic variation for any of the three traits in any environment. I did find significant maternal variances for all traits, and in some cases these differ between environments. These results indicate that the evolution of larval mass, body size and development time is only possible through maternal and offspring interactions.

4.2 INTRODUCTION

Natural selection acts on phenotypic variation (Wilson, 2008); but what causes the accumulation and maintenance of phenotypic variation? Phenotypic variation is essential for evolution, as different phenotypes confer differential fitness which is selected for or against by natural selection. Selection, however, does not differentiate between the origin of phenotypic variation, be it genetic or environmental. It is therefore important to understand the causes of phenotypic variation as it has large implications for the subsequent evolution of a trait (Falconer and Mackay, 1996; Roff, 2012).

Genetic variation underlies much of the phenotypic variation in nature. This is not only the case for discrete traits where one allele maps onto one phenotype, but also for quantitative traits, where many genes of small effect add together to cause a continuous distribution of phenotypic variation (Lynch and Walsh, 1998). The total phenotypic variance in a trait (V_P) is made up of the additive genetic component (V_A), the environmental component (V_E) and residual variation (V_R), where the ratio V_A/V_P equals the narrow-sense heritability (h^2), the value that determines the evolutionary potential of a trait. The Breeder's equation ($R = h^2S$) determines the response to selection (R) by multiplying the narrow-sense heritability (h^2) with the strength of selection (S, Lush, 1937). As a ratio, however, the heritability changes depending on both the phenotypic variance and the additive genetic variance. Therefore merely measuring the heritability may not be enough to get a good understanding of the evolvability of a trait (Houle, 1992), which explains why the Breeder's equation rarely predicts evolution in the wild (Merilä, Kruuk and Sheldon, 2001; Morrissey, Kruuk and Wilson, 2010).

A trait's heritability also changes depending on the environment, the population measured, and the time it was estimated. Environments are stochastic, rarely homogenous, and are highly influential in the expression of additive genetic variation (Charmantier and Garant, 2005; Hoffmann and Merilä, 1999; Rowiński and Rogell, 2017). An increase in heritability due to a change in the environment can result from an increase in additive genetic variation, which means more of the phenotypic variation is now explained by the genetic contribution, increasing that trait's ability to respond to selection. Estimating V_A is therefore a potential way of estimating cryptic genetic variation (McGuigan and Sgro, 2009), which is now thought to have a prominent role in adaptive evolution (Gibson and Dworkin, 2004; Hayden, Ferrada and Wagner, 2011; Le Rouzic and Carlborg, 2008; Masel, 2006; McGuigan and Sgro, 2009; Paaby and Rockman, 2014). Cryptic genetic variation is genetic variation present in the genome that does not currently contribute to phenotypic variation. It only contributes to the phenotype when there is a change in the genetic background (Rutherford, 2003) or a change in the environment (McGuigan et al., 2011). As such, cryptic genetic variation could be considerable and ready to fuel adaptive evolution should it be revealed.

Identifying factors that contribute to the accumulation and release of cryptic genetic variation would allow a greater understanding of how adaptive evolution could unfold. Parental care could be one such factor. While they care and provide resources for their developing offspring, parents buffer their offspring from selection, hiding potentially deleterious mutations from selection (Pilakouta et al., 2015; Snell-Rood et al., 2016). The environment in which individuals develop includes their social environment, variation in which is itself partly attributable to

that individual's parents and siblings. The gene for a trait which is half influenced by social partners and half by the gene itself, is under relaxed selection. Selection is acting on both genes in the individual and their social partner, which lessens its strength (Wade, 1998). This is why social genes are expected to have greater levels of polymorphisms (Linksvayer and Wade, 2009). Snell-Rood et al. (2016), in their recent review, called this the *relaxed selection* hypothesis, whereby genes are under selection in the social environment provided by parents, and purifying selection is relaxed on deleterious mutations in an environment without parents (Kawecki, 1994; Pilakouta et al., 2015). Two other hypotheses were presented by Snell-Rood et al. (2016): the *stress* hypothesis, stating that new mutations are under greater selection pressure, which parents mitigate; and, the *compensation* hypothesis, where parents who provide direct care can compensate for offspring deficiencies. Their data using *Onthophagus* dung beetles appear to support both the *stress* and *relaxed selection* hypotheses.

The social environment is also an environment that can lead to the build up and maintenance of phenotypic and genetic variation through genotype-by-environment interactions (G × E, Lynch and Walsh, 1998). G × E interactions occur when the same genotype has different phenotypes in different environments, and has received attention in crop and animal breeding (Falconer and Mackay, 1996) and sexual selection research (Ingleby, Hunt and Hosken, 2010). G × E interactions lead to the build up genetic variation (Gillespie and Turelli, 1989), especially if the reaction norms cross and the rank order of genotypes differs between environments. G × E interactions can also occur without crossing reaction norms or a change in rank order if the genetic variation in one environment is greater than the variation in the other environment. One possible way for this to occur is if the second environment reduces a possible constraint on achieving a phenotype which is less constrained by the environment, and therefore more determined by the underlying genes. Understanding how the social environment contributes to evolution involves partitioning sources of variation in the phenotype. Specifically, not only the additive genetic component contains genes. The environment consists of the maternal genes, and taking that into account, the residual variation can be interpreted as the social environment created by an individual's siblings.

Figure 4.1 graphically outlines how to decompose phenotypic variation to different levels, and indicates the implications for the evolution of a trait. Phenotypic variation can be equal in different environments (Figure 4.1a), but that phenotypic variation can be driven by a variety of causes. Figure 4.1b decomposes the variation to the group level. Groups can be a particular genotype, in which case Figure 4.1b would indicate additive genetic variance (V_A), or an individual family, in which case it would show the variance attributable to the mother and

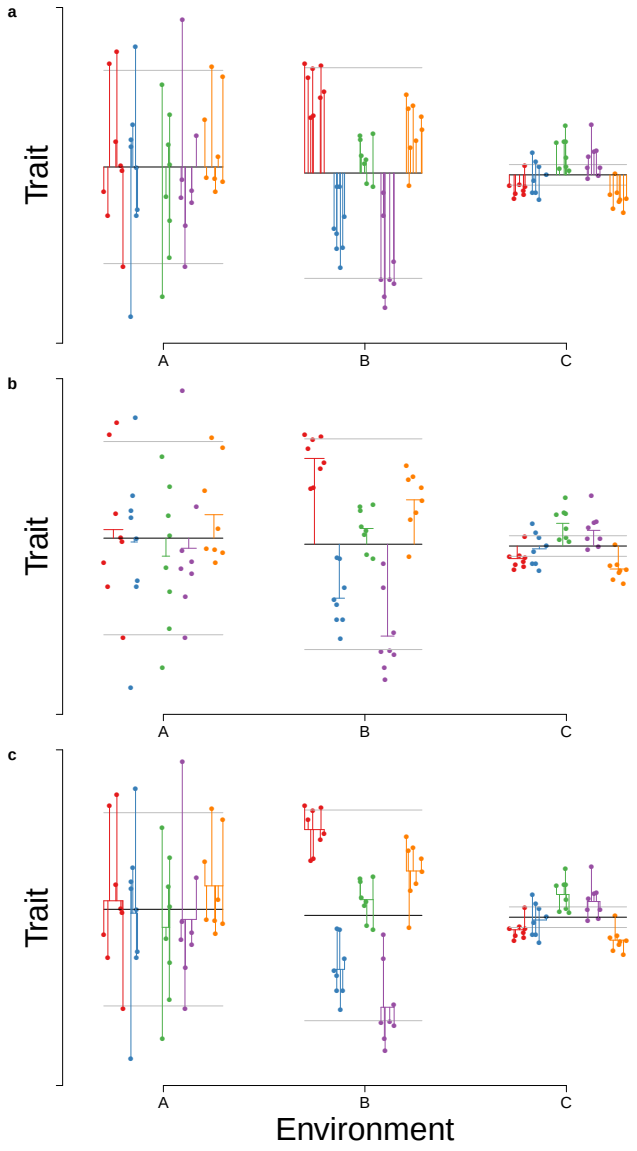


Figure 4.1 | A graphical representation of how phenotypic variation can be attributed to different sources. All three plots show the same data, which consists of eight individuals from five groups (as depicted by the different colours) measured in three environments, A, B, and C. The population mean in each environment is shown as a horizontal black line, the populations variance is marked by horizontal grey lines, and the group means are shown as horizontal lines in the colour of the group in question. **a** The top plot shows the overall population variation. Trait variation is equal in environments A and B, with reduced variation in environment C. **b** The middle plot shows how variation is attributed to the group, measured by the deviation of each group mean from the population mean. Variance attributable to the group is equal in environments A and C, and greatest in environment B. **c** The bottom plot shows the residual variation (i. e. the variance around the group mean). This within group variance is smallest in environment C and greatest in environment A.

permanent environment. The within-group or residual variation is shown in Figure 4.1c. It is only through estimating the relative compnents of phenotypic variation that we can determine how a trait might evolve under selection. High maternal (between group variance where the group is the dam, Figure 4.1b) or sibling variance (within group variance, Figure 4.1c) indicate a trait can likely evolve through indirect genetic effects (Rauter and Moore, 2002), whilst large additive variance (between group variance where the group is a particular genotype, Figure 4.1b) allows the evolution through traditional individual selection.

Burying beetles (*Nicrophorus* spp.) are a good system to ask how different social and resource environments affect the expression of phenotypes and the extent of variation in those phenotypes. With the right experimental design, the source of this variation can also be determined, which the burying beetle allows through easy laboratory manipulation. I assessed how three traits were influenced in four environments, created by combining two social environments and two resource environments, in a fully factorial design. The social environment was either Full Care, where the mother was present to provide care for her offspring, or No Care, where the mother was not present to look after her offspring. The manipulation of the social environment follows Rauter and Moore (2002), where the authors estimated additive genetic variation in the same two environments in a different burying beetle species (*N. pustulatus*). Rauter and Moore (2002) did not find differences in additive genetic variation in morphological traits between the two environments. One hypothesis is that the carcass size imposes a constraint on achieving a phenotype that is not overwhelmingly influenced by the environment. To counter such potential constraint, I introduced a second dimension: the size of the carcass breeding resource, which follows Andrews, Kruuk and Smiseth (2016). Furthermore, I have previously shown that both the social environment and the size of the breeding resource likely play an important role in the diversification of the burying beetles (Chapter 3). The four environments therefore were: Full Care on a Large Carcass; Full Care on a Small Carcass; No Care on a Large Carcass; and, No Care on a Small Carcass.

The first hypothesis I tested was whether the social environment and resource environment interact to determine offspring phenotype. I predicted that when parents are present to care for their offspring, their offspring will attain a larger mass and therefore larger adult size, and this is independent of the size of the breeding resource as parental care would mask any effects of the environment. Without parents, I predicted that the Large carcass would relax constraint on offspring phenotype and result in larger offspring (Bartlett and Ashworth, 1988; Scott and Traniello, 1990; Smiseth and Moore, 2002a; Smiseth et al., 2014; Trumbo, 1991). With similar logic, the second hypothesis I tested was whether parental care buffers genetic variation from selection, which would increase the

quantities of cryptic genetic variation within the population (Snell-Rood et al., 2016; Wade, 1998). One way to test for the release of cryptic genetic variation would be to estimate additive genetic variation when the parents are present and when parents are absent (McGuigan and Sgro, 2009). With the mother caring for her offspring, the genetic contribution to offspring phenotype is reduced and so yield a low heritability through low additive genetic variation. Removing parental care unveils the underlying genes which then contribute to the phenotype. I predicted this will only occur when the carcass is large and does not impose a constraint on offspring phenotype. It is only in the No Care Large Carcass environment where the lack of constraint on offspring phenotype and the lack of a masking effect by parents would result in significant additive genetic variation contributing to the offspring phenotype.

Genetic variation also accumulates through G × E interactions, where multiple environments favour variation in strategies, as no single strategy or genotype is best in all environments. I therefore tested how the mean phenotype of dams changed across environments, where I predicted that both the rank order (e. g. the size of offspring they produce in one environment relative to the others in that environment) and sizes of offspring differ across environments, both indicate G × E interactions. As well as partitioning out phenotypic variation to genetic contributions, I also parsed out contributions due to the maternal environment and residual variance. I predicted the variation attributable to dam and residual effects will shift between environments, ultimately influencing the mechanisms by which each trait will evolve in each environment (Bijma and Wade, 2008).

The mixture of traits I chose to measure reflect aspects of the burying beetle that are closely tied to their fitness, and linked to the radiation of the genus (Chapter 3). Phenotypes are not independent and can evolve in tandem, or constrain the evolution of each other. The **G** matrix provides estimates of the genetic (co)variances among traits and determines how a suite of traits can potentially shift under selection for one or many traits (Walsh and Blows, 2009). As larger species tend to be univoltine and smaller species can have up to two generations a year (Scott, 1998b), I predict a negative genetic correlation between body size, both at the larval and adult stage, and the speed of development where smaller individuals develop in a shorter time. This negative correlation may have reinforced the speciation process, with a shift in development time reducing temporal competition between species (Chapter 3, Scott, 1998b).

4.3 METHODS

4.3.1 *Experimental design*

The individual beetles (*Nicrophorus vespilloides*) used for this experiment were derived from two populations that had been bred in the lab for 11 generations under a Full Care environment, where parents were allowed to care for their offspring after hatching. The populations were founded in 2015 from individuals from four wild populations collected at different sites in Cambridgeshire. They were interbred for one generation, with each F_1 family contributing to both populations (see Schrader et al., 2017, for more details). I performed a full-sib/half-sib split-family quantitative genetics experiment (Lynch and Walsh, 1998) to estimate the genetic variances and covariances of and between several phenotypic traits, both within and across four distinct environments. The experiment was done in a fully-factorial design consisting of two social environments, Full Care (F) and No Care (N), and two resource environment, a large mouse carcass (L) or a small mouse carcass (S). Through the design of the experiment, the Full Care environment consisted of maternal only care. Previous work has shown that females consistently perform the majority of post-hatching parental care, and male care makes no material difference to larval performance in the lab (Parker et al., 2015; Walling et al., 2008).

A single male was mated with four virgin females over the course of four days, one female per day. The male and female were placed in a breeding box for 24 hours with soil and a small amount of mince. Once the male was removed, the female was given a recently defrosted small mouse (10–12 g) upon which to breed. At the same time, an equivalent number of donor virgin females were given a large mouse each (40–50 g) to prepare. Focal females were removed at ~ 53 hours, once carcass preparation and egg laying had been completed (Boncoraglio and Kilner, 2012). Donor females were left until ~ 70 hours after being provided with the mouse, as carcass preparation takes longer with a larger carcass (Trumbo, 1992). The focal female was then removed from her breeding box into another breeding box with fresh soil containing no eggs, and a new carcass (i. e. not the one she herself prepared). At this stage I haphazardly determined the first two environments for each female (Figure 4.2). The focal female was either given a box with a Small or a Large carcass to raise her offspring. At the same time, there was another fresh breeding box with the alternate size of carcass, and it was on this resource that the focal female's offspring would be reared without maternal care. In the second breeding bout, the treatments were reversed, so that each female would end up contributing a brood in each of the four treatments (Figure

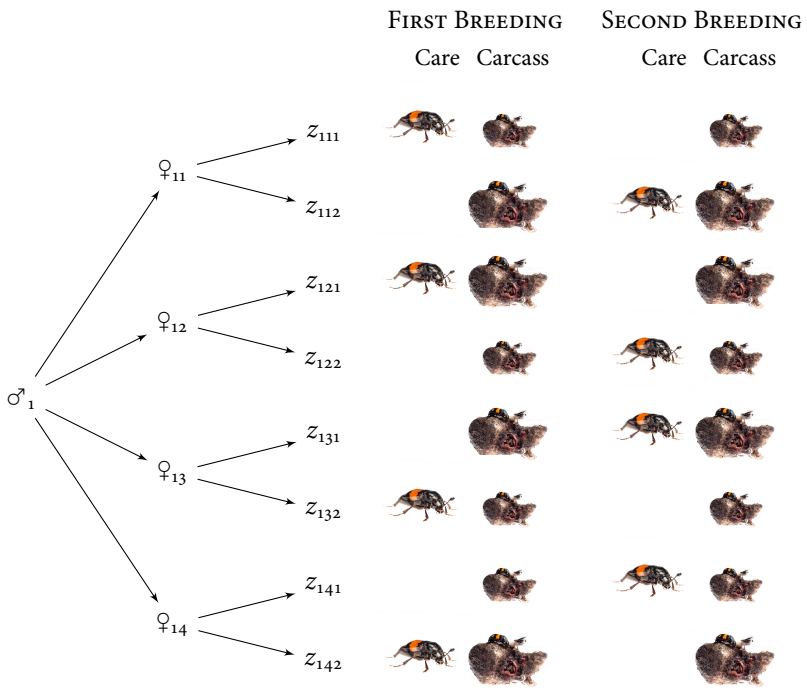


Figure 4.2 | The design of the quantitative genetic experiment. One male was mated with four different virgin females. The females then bred twice. The four environments are designated by the presence or absence of a female beetle in the first column, and the size of the carcass shown in the second column. In their first breeding event, the female's offspring were split and distributed evenly across two of the four total environments (the first two columns), with z indicating at most 12 offspring from that female. For example, the offspring of $\female_{11}$ are first split into the Full Care Small Carcass environment and the No Care Large Carcass environment. Note that both environments cannot replicate any aspect of the other environments in one breeding event (i. e. there are not two mothers for both environments to be Full Care in one breeding event). In the second breeding event, the other two environments not used in the first breeding event are used. Again, in the case of $\female_{11}$, these are No Care Large Carcass and Full Care Small Carcass, displayed in the second two columns.

4.2). This design was replicated for 54 males across two populations that were bred a week apart.

The breeding box that contained the focal female's eggs was monitored three times a day for larval hatching. Once the larvae started hatching, I transferred the larvae to either the box with their mother, or without. Transferring the larvae was done haphazardly, but ensuring even distribution across both environments. I added larvae until a maximum of 12 larvae were on the carcass. The breeding boxes with the larvae in were checked three times a day. Larval dispersal was determined when two or more larvae were seen crawling away from the remains of the carcass (Rauter and Moore, 2002). At dispersal, I weighed the larvae

individually and placed them into an eclosion box in the same order as I weighed them. In this way, I was able to relate larval mass at dispersal to the size the individual attained at eclosion.

Once in the eclosion boxes, the beetles were checked three times a day until development was complete, which was deemed to have occurred when adults were fully melanised. Once the family had completed development, each individual was anaesthetised with CO_2 and photographed. The size of the adult was then calculated from the photograph using a custom MATLAB script (see Chapter 2 and the Appendix). In total, I had data on the larval mass, development time, and adult size of each individual.

4.3.2 *Power analysis*

I performed a power analysis using the expected sample size from the number of half-sib males to estimate the likely power I will have at detecting significant heritability for one trait using this pedigree. I followed guidance from Morrissey et al. (2007) and used the `pedantics` package (Morrissey, 2014; Morrissey and Wilson, 2010) in R 3.3.0 (R Development Core Team, 2016). I estimated the power for a phenotypic trait of a known heritability which I varied from 0 to 0.2 with 0.01 increments using the `phensim` function. The analysis was completed 1000 times for each heritability, with the power calculated as the proportion of these 1000 analyses that yielded a significant heritability. The power analysis estimates that the power of the current experiment would have an 80% chance of detecting heritabilities of 0.1 (Table 4.1).

Table 4.1 | The estimates of power given the sample size of the quantitative genetics experiment outlined above for a realistic range of heritabilities.

Heritability	Power	Heritability	Power
0.00	0.061	0.11	0.839
0.01	0.074	0.12	0.872
0.02	0.121	0.13	0.906
0.03	0.196	0.14	0.937
0.04	0.274	0.15	0.963
0.05	0.389	0.16	0.972
0.06	0.478	0.17	0.975
0.07	0.582	0.18	0.984
0.08	0.674	0.19	0.992
0.09	0.743	0.20	0.991
0.10	0.798		

4.3.3 *Statistical analysis*

I analysed the data at the phenotypic level using `lme4` in `R` 3.3.0 (R Development Core Team, 2016). I fit the interaction between the social environment and the size of the carcass with the number of larvae left surviving in the brood as a covariate. The unique ID of each sire and dam were included as random terms, as well as the two blocks of the experiment. I compared models with and without the interaction to determine the significance of terms.

Quantitative genetic approaches make use of known relatedness between individuals to estimate genetic parameters (Falconer and Mackay, 1996; Lynch and Walsh, 1998). Such information, collected through genotyping approaches in wild populations and breeding designs in the lab, can be fed into an animal model (Kruuk and Hadfield, 2007; Kruuk, 2004). This is a powerful approach, both in field studies and lab studies, that utilises the pairwise relatedness coefficients between individuals to disentangle genetic and common environment contributions to the phenotype (Kruuk and Hadfield, 2007; Lynch and Walsh, 1998; Wilson et al., 2010). For this experiment, I used a breeding design that created half-sibling families which share a sire. By comparing half-siblings with one another, I could tease apart the genetic contribution to phenotypic variation via the shared paternal genetic contribution. The common environment contribution to the phenotype was examined by comparing full-siblings.

To incorporate the pedigree data, I analysed data for each treatment separately using the package `ASreml-R` 3.0 (Butler et al., 2017) in `R` 3.3.0 (R Development Core Team, 2016). Models included a fixed effect of the number of larvae surviving per brood (mean-centred), a random effect of brood ID to estimate variance due to permanent environmental (including maternal) effects, and a random effect of the pedigree term to estimate the additive genetic variance. I was unable to partition variance due to maternal effects from that of the permanent environment because no females had multiple broods within a single environment. I then tested the significance of the additive genetic variance in adult size by comparing models with and without the pedigree term using a likelihood ratio test. I estimated χ^2_{nDF} as twice the difference in model log likelihoods. Given that I was testing the effect of a single variance component (nDF = 1), I assumed the test statistic to be asymptotically distributed as an equal mix of χ^2_0 and χ^2_1 (Visscher, 2006). The heritability was calculated as V_A/V_P, where V_P is the sum of the variance components (additive genetic, permanent environment, and residual) from the model, having conditioned on the fixed effects. I used Wald F-tests to estimate the significance of fixed effects.

As there was no evidence for additive genetic variance for any trait in any environment, I did not estimate the additive genetic covariances across environments

(which are used in determining the presence of G × E interactions). Instead, I moved onto looking at the effect of the environment on the (co)variation among dams. The 'dam' term in these models includes both the maternal genetic and permanent environment effects of a single brood. As each dam had only a maximum of one brood in each environment, I was unable to disentangle the two, and so results for this section should be treated with that in mind. However, by assaying a dam's broods across multiple environments, I was able to estimate the dam-related covariance. To examine the existence and nature of the dam × environment interaction across the four environments, I fit a series of models to the data and used likelihood ratio tests to determine which one fit best. For each model, I included the pedigree term as an identity matrix (such that I estimated any additive genetic effects in each environment but did not fit their covariance), and fit the residual variation as an unstructured covariance matrix. Model 1 did not fit dam identity in the model, and so acted as the null model where the dam does not explain any of the variation. Model 2 included the dam term, but did not allow the variance of the dams to change (i. e. the variance explained by dams is constrained to be the same in both environments). Comparing model 2 to model 1 allowed estimates of the existence of significant dam-related variance. The third model allowed the dam variance to differ across environments (noting that significant differences in the variance across environments would demonstrate a form of dam × environment interaction). Model 4 extended the third model by fitting the dam-related covariance across environments. Finally, to test whether there were dam × environment interactions due to changes in the rank order, model 5 allowed dam variances to differ but fixed the correlation across environments to +1 (i. e. a perfect correlation across environments). If model 4 was a better fit than model 5, then significant dam × environment exist because it means that the rank order of dams is not consistent across environments.

To scrutinise the level at which variation is maintained, I used multivariate analyses within environments for all three traits to ask how phenotypic values covary between traits, and how this variation is attributable to the dam or within the brood. All traits were scaled by the global standard deviation (including all environments, to maintain environment-specific differences in both the mean and the variance) to make them comparable within, and between, environments, in addition to helping with mutivariate model fitting. I also multiplied development time by −1, such that positive relationships between all traits would generate variation on a single axis of offspring 'performance' (i. e. where heavier and larger offspring also develop faster). I first used models with no random effects, estimating the phenotypic variance in a covariance among the three traits of interest in each environment (after controlling for the fixed effects of the number of larvae within each brood and the exact mass of the carcass). Secondly,

I fitted models that included random effects of the pedigree (estimating only the variance for each trait, no covariances between them) and the dam (estimating both the variance and covariance at this level). From this model, I was able to partition the phenotypic (co)variances (again, controlling for the fixed effects of the number of larvae and mass of the carcass) into among-dam and within-brood components. At the among-dam level, I estimated how much variation in each trait was due to differences among dams, as well as the covariance structure between these traits. This is the variation explained as outlined in Figure 4.1b where the group is the ID of the dam. The residual (co)variance can effectively be interpreted as within-brood variance, given that the lab in which the experiment was conducted was stable, offering very little in terms of deviance to add to the residual variance (and noting again my inclusion of fixed effects of the number of larvae within each brood, and the exact mass of the carcass as statistical controls within carcass treatments). Again, in reference to Figure 4.1, this represents the bottom panel when the group is the family. The within-brood variance is therefore the variation of offspring phenotypes around the mean of their mother, after controlling for fixed effects and the pedigree variance. Within-brood covariances demonstrate the relationships between traits within broods.

4.4 RESULTS

4.4.1 *Phenotypic results*

Larval mass was weakly, but significantly, influenced by the interaction between parental care and the size of the carcass (estimate ± standard error = −0.0057 ± 0.0024 g, $\chi^2_1 = 5.56, P = 0.018$); offspring that developed on a small carcass without parental care were lighter than those that developed on a large carcass without parents (Figure 4.3). As expected, there was a large effect of care on larval mass, with individuals receiving care tending to be heaver on average (−0.0398 ± 0.0018 g, Figure 4.3).

Development time was also significantly affected by the interaction of carcass mass and parental care, but the effect was again weak (−6.83 ± 2.54 hrs, $\chi^2_1 = 7.25, P = 0.007$). Development time was the same when the mother was present independent of carcass size, but when the mother was not present, offspring developed faster when they were reared on a small carcass (Figure 4.4). This result appears consistent with the fact that smaller offspring develop faster (Rauter and Moore, 2002), and so is expected following on from the similar pattern of larval mass.

Larval mass accurately predicts adult body size, which is why there is also a significant and weak interaction between carcass and care on adult pronotum

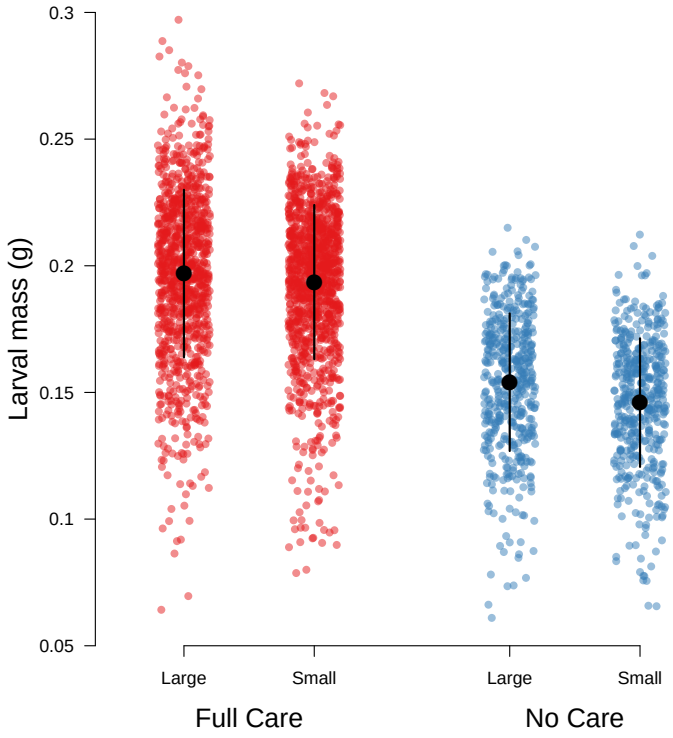


Figure 4.3 | Larval mass attained in each environment. The Full Care environments are in red, and the No Care environments are in blue. 'Large' and 'Small' refer to the carcass size. Black points indicate the trait mean in the environment, and the black error bars denote standard deviation.

width (−0.077 ± 0.032 mm, χ^2_1 = 5.92, P = 0.015). Smaller adults resulted from being reared without parents generally, but were even more likely to be small when they had been raised on a small carcass (Figure 4.5).

4.4.2 *Genetic additive variance in all four environments*

The next step was to determine whether any of the four environments resulted in the expression of significant additive genetic variation. I found no significant V_A for larval mass in the No Care Small Carcass (Log likelihood (LL) = 0.32, P = 0.285), Full Care Large Carcass (LL = 2.49, P = 0.057), or No Care Large Carcass (LL = 0.43, P = 0.418, Table 4.2). I did find significant heritability of larval mass in the Full Care Small Carcass environment (LL = 4.35, P = 0.018), but the error associated with the estimate of V_A was large, and so this finding should be treated with some level of scepticism.

The results were similar for development time (Table 4.3), which contrasts with the results for development time ("in pupal chamber") obtained by Rauter and Moore (2002). All estimates for V_A in each environment did not differ from

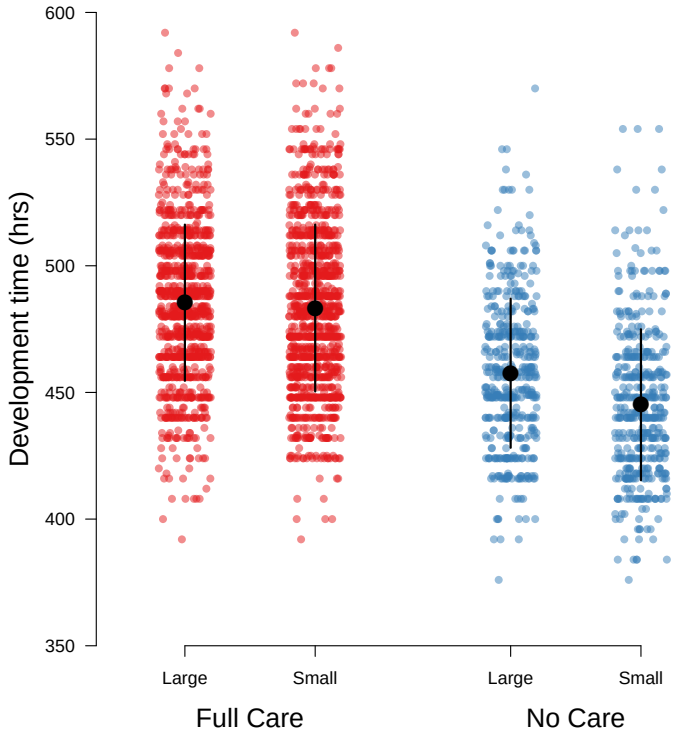


Figure 4.4 | Development time in each environment. The Full Care environments are in red, and the No Care environments are in blue. 'Large' and 'Small' refer to the carcass size. Black points indicate the trait mean in the environment, and the black error bars denote standard deviation.

zero (Full Care Small Carcass: LL = 0.32, P = 0.285; No Care Small Carcass: LL = -1.31×10^{-5}, P = 0.5; Full Care Large Carcass: LL = 0.03, P = 0.423; No Care Large Carcass: LL = -5.56×10^{-5}, P = 0.5).

There was also no significant additive genetic variance for adult pronotum width (Table 4.4). As with development time, there was no difference between environments, where all estimates overlapped with zero (Full Care Small Carcass: LL = 0.46, P = 0.249; No Care Small Carcass: LL = 0.03, P = 0.428; Full Care Large Carcass: LL = 0.11, P = 0.370; No Care Large Carcass: LL = 1.77, P = 0.092).

4.4.3 *Permanent environment/maternal variance across all four environments*

I found that there was significant maternal variance for all traits in all environments. Larval mass (Table 4.2) had significant maternal variation in all environments: Full Care Small Carcass (LL = 9.58, $P < 0.001$), No Care Small Carcass (LL = 3.03, P = 0.041); Full Care Large Carcass (LL = 14.52, $P < 0.001$); and, No Care Large Carcass (LL = 8.46, P = 0.002).

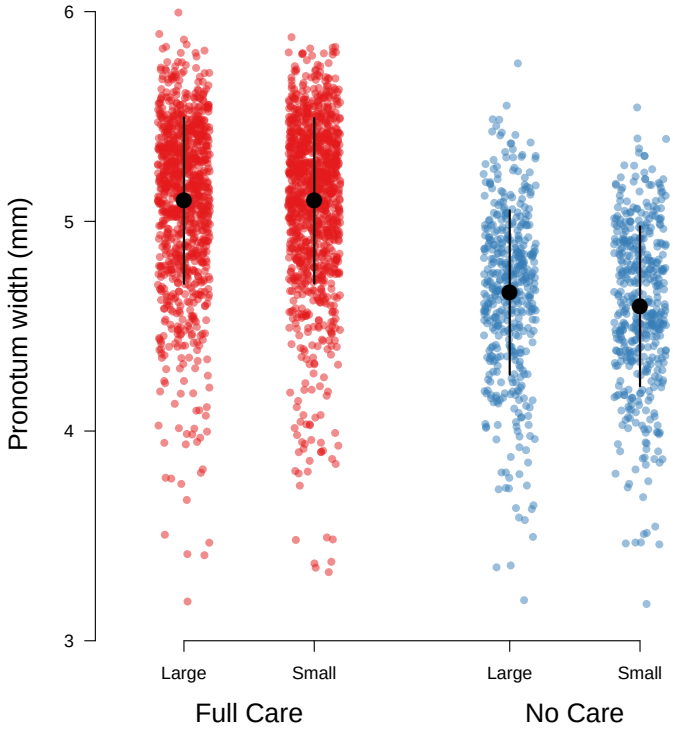


Figure 4.5 | Pronotum width in each environment. The Full Care environments are in red, and the No Care environments are in blue. 'Large' and 'Small' refer to the carcass size. Black points indicate the trait mean in the environment, and the black error bars denote standard deviation.

Table 4.2 | The variance components for larval mass in *N. vespilloides*. The heritability (h^2), additive genetic variance (V_A), variance due to a common environment (V_{PE}), and residual variance (V_R) in all environments are shown. All values given are ± a standard error and are given in units of milligrams.

	h^2	V_A	V_{PE}	V_R
Full Care Small Carcass	0.21 ± 0.12	0.23 ± 0.13	0.19 ± 0.07	0.67 ± 0.07
No Care Small Carcass	0.20 ± 0.34	0.16 ± 0.27	0.24 ± 0.14	0.41 ± 0.14
Full Care Large Carcass	0.21 ± 0.15	0.24 ± 0.17	0.34 ± 0.09	0.58 ± 0.09
No Care Large Carcass	0.06 ± 0.27	0.05 ± 0.24	0.35 ± 0.13	0.47 ± 0.12

This was also the case for development time (Table 4.3: Full Care Small Carcass (LL = 16.09, $P < 0.001$), No Care Small Carcass (LL = 9.33, $P = 0.001$); Full Care Large Carcass (LL = 20.60, $P < 0.001$); and, No Care Large Carcass (LL

Table 4.3 | The variance components for development time in *N. vespilloides*. The heritability (h^2), additive genetic variance (V_A), variance due to a common environment (V_{PE}), and residual variance (V_R) in all environments are shown. All values given are ± a standard error. Values are given in units of hours.

	h^2	V_A	V_{PE}	V_R
Full Care Small Carcass	0.08 ± 0.14	85.39 ± 154.64	337.76 ± 88.95	669.71 ± 83.13
No Care Small Carcass	0.00 ± 0.00	0.00 ± 0.00	266.60 ± 63.78	642.36 ± 47.58
Full Care Large Carcass	0.02 ± 0.14	22.91 ± 135.89	367.04 ± 84.19	563.96 ± 72.54
No Care Large Carcass	0.00 ± 0.00	0.00 ± 0.00	486.92 ± 88.87	450.36 ± 33.89

Table 4.4 | The variance components for pronotum width in *N. vespilloides*. The heritability (h^2), additive genetic variance (V_A), variance due to a common environment (V_{PE}), and residual variance (V_R) in all environments are shown. All values given are ± a standard error. Values are given in units of millimetres.

	h^2	V_A	V_{PE}	V_R
Full Care Small Carcass	0.08 ± 0.12	0.01 ± 0.02	0.05 ± 0.01	0.12 ± 0.01
No Care Small Carcass	0.05 ± 0.30	0.01 ± 0.04	0.05 ± 0.02	0.09 ± 0.02
Full Care Large Carcass	0.04 ± 0.12	0.01 ± 0.02	0.04 ± 0.01	0.10 ± 0.01
No Care Large Carcass	0.40 ± 0.33	0.06 ± 0.05	0.04 ± 0.02	0.05 ± 0.02

= 17.88, $P < 0.001$); and also pronotum width (Table 4.4): Full Care Small Carcass (LL = 16.22, $P < 0.001$), No Care Small Carcass (LL = 6.05, $P = 0.007$); Full Care Large Carcass (LL = 16.71, $P < 0.001$); and, No Care Large Carcass (LL = 3.16, $P = 0.04$).

By comparing model 2 with model 3, I can ask if more phenotypic variation was partitioned out to the maternal/permanent environment. In most cases there was no evidence to suggest maternal variance differs between the pairs of environments (comparing across care environments on small and large carcasses, and across carcass regimes when parents were absent, all $P > 0.07$). When comparing maternal variances across carcass sizes when parents were present to care for their offspring, I found that more phenotypic variation was attributable to the dam when on a large carcass for larval mass ($P = 0.05$) and development time ($P = 0.007$), but not pronotum width ($P = 0.71$).

4.4.4 *Phenotypic (co)variances between traits across all four environments*

Tables 4.5, 4.6 and 4.7 display the phenotypic, maternal, and residual variances respectively, of each trait in each environment and how they covary. The phenotypic variances are on the diagonals, and appears larger in the Full Care environments independent of Carcass size, but this effect appears to be driven by an increase in variation of larval mass (Table 4.5). The covariance between larval mass and adult size is strong in all environments, but the covariance between development time and the two morphological traits is weak. This covariance is greater when parents are absent on a large carcass, than when they are present on a large carcass, which may indicate that parental care alters how resources are allocated within individuals when resources are not limited.

The maternal (co)variance matrix (Table 4.6) aligns with the results from the previous section, in that greater variance is attributable to the dam on large carcasses generally, but more particularly when the dam is present. The dam variances for the No Care Large Carcass environment were also high, but did not differ when compared between environments. The variation remaining within a brood (the residual variance, Table 4.7) is much larger for both morphological traits when parents are present. This means that each brood is more variable in terms of larval mass and adult size when the dam is present, independent of carcass size.

Table 4.5 | The phenotypic variance-covariance-correlation matrix of all traits in all four environments. The variance of each trait is on the diagonal in bold, the phenotypic covariance between traits is below the diagonal in italics, and the phenotypic correlation between traits is above the diagonal. Standard errors are in below the estimate in parentheses.

Full Care Small			
	Mass	Dev	Pronotum
Mass	**0.728 (0.027)**	0.323 (0.028)	0.766 (0.011)
Dev	*0.261 (0.024)*	**0.9 (0.036)**	0.122 (0.028)
Pronotum	*0.621 (0.027)*	*0.11 (0.026)*	**0.903 (0.035)**

Full Care Large			
	Mass	Dev	Pronotum
Mass	**0.773 (0.029)**	0.288 (0.027)	0.697 (0.015)
Dev	*0.222 (0.023)*	**0.767 (0.032)**	0.122 (0.028)
Pronotum	*0.527 (0.025)*	*0.029 (0.022)*	**0.742 (0.03)**

No Care Small			
	Mass	Dev	Pronotum
Mass	**0.524 (0.033)**	0.363 (0.042)	0.807 (0.016)
Dev	*0.23 (0.033)*	**0.768 (0.052)**	0.132 (0.047)
Pronotum	*0.523 (0.038)*	*0.103 (0.038)*	**0.8 (0.035)**

No Care Large			
	Mass	Dev	Pronotum
Mass	**0.565 (0.034)**	0.485 (0.037)	0.762 (0.019)
Dev	*0.317 (0.034)*	**0.757 (0.051)**	0.211 (0.045)
Pronotum	*0.496 (0.036)*	*0.11 (0.026)*	**0.751 (0.049)**

Table 4.6 | The maternal variance-covariance-correlation matrix of all traits in all four environments. The variance of each trait is on the diagonal in bold, the maternal covariance between traits is below the diagonal in italics, and the maternal correlation between traits is above the diagonal. Standard errors are in below the estimate in parentheses.

	Full Care Small				Full Care Large		
	Mass	Dev	Pronotum		Mass	Dev	Pronotum
Mass	**0.197 (0.029)**	0.413 (0.087)	0.749 (0.046)	Mass	**0.303 (0.039)**	0.29 (0.087)	0.806 (0.038)
Dev	*0.104 (0.027)*	**0.319 (0.046)**	0.122 (0.028)	Dev	*0.089 (0.03)*	**0.313 (0.043)**	−0.065 (0.102)
Pronotum	*0.164 (0.028)*	*0.031 (0.029)*	**0.111 (0.101)**	Pronotum	*0.211 (0.032)*	*−0.017 (0.027)*	**0.225 (0.033)**

	No Care Small				No Care Large		
	Mass	Dev	Pronotum		Mass	Dev	Pronotum
Mass	**0.218 (0.045)**	0.497 (0.12)	0.842 (0.045)	Mass	**0.247 (0.064)**	0.567 (0.092)	0.735 (0.06)
Dev	*0.115 (0.04)*	**0.246 (0.058)**	0.283 (0.147)	Dev	*0.185 (0.049)*	**0.433 (0.079)**	0.275 (0.124)
Pronotum	*0.215 (0.048)*	*0.077 (0.044)*	**0.298 (0.063)**	Pronotum	*0.212 (0.048)*	*0.105 (0.053)*	**0.337 (0.064)**

Table 4.7 | The residual variance-covariance-correlation matrix of all traits in all four environments. The variance of each trait is on the diagonal in bold, the residual covariance between traits is below the diagonal in italics, and the residual correlation between traits is above the diagonal. Standard errors are in below the estimate in parentheses.

	Full Care Small				Full Care Large		
	Mass	Dev	Pronotum		Mass	Dev	Pronotum
Mass	**0.533 (0.021)**	0.267 (0.029)	0.761 (0.013)	Mass	**0.48 (0.019)**	0.246 (0.03)	0.651 (0.018)
Dev	*0.151 (0.018)*	**0.604 (0.026)**	0.096 (0.031)	Dev	*0.119 (0.016)*	**0.486 (0.022)**	0.059 (0.032)
Pronotum	*0.441 (0.021)*	*0.059 (0.019)*	**0.629 (0.026)**	Pronotum	*0.33 (0.018)*	*0.03 (0.016)*	**0.536 (0.024)**

	No Care Small				No Care Large		
	Mass	Dev	Pronotum		Mass	Dev	Pronotum
Mass	**0.33 (0.024)**	0.339 (0.048)	0.788 (0.002)	Mass	**0.334 (0.023)**	0.438 (0.045)	0.789 (0.02)
Dev	*0.145 (0.024)*	**0.55 (0.041)**	0.086 (0.053)	Dev	*0.161 (0.022)*	**0.406 (0.031)**	0.183 (0.052)
Pronotum	*0.324 (0.027)*	*0.046 (0.029)*	**0.215 (0.038)**	Pronotum	*0.310 (0.025)*	*0.079 (0.023)*	**0.462 (0.034)**

4.4.5 *Dam × environment interactions*

Significant dam × environment interactions were obtained for all traits between all environments (Figures 4.6, 4.7, and 4.8). In every comparison between two environments, dams varied in their rank order and slopes. Thus, if a dam produced

large offspring, or offspring that developed faster in one environment it did not mean necessarily she produced offspring that were small or slow developers in the other environment. The model that best fit the data was model 4: dam-related variance was allowed to differ across environments, and the covariance was estimated (and did not represent a perfect correlation across environments). The existence of dam × environment interactions across each pair of environments, for each traits can be seen clearly in (Figures 4.6, 4.7, and 4.8). See figure captions for the statistic associated with comparing model 4 with model 5.

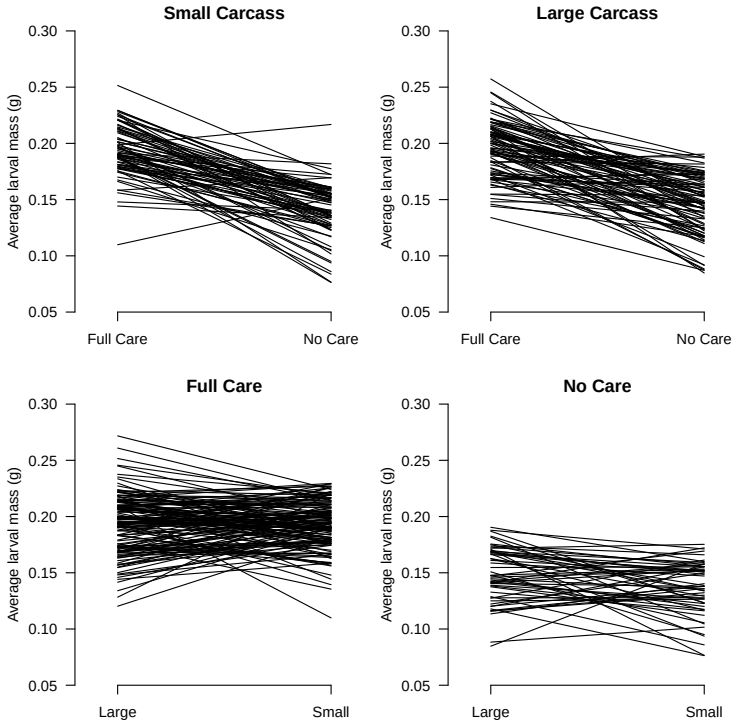


Figure 4.6 | Reactions norms of larval mass between all four environments. The top row of plots shows the reaction norm between the two parental care environments on either a Small carcass (left) or a Large carcass (right). The bottom row shows the reaction norm for each dam across carcass environments in Full Care (left) and No Care (right). There was significant dam × environment interaction across care environments on a small (P = 0.022) and a large carcass (P = 0.002), and across carcass environments with care (P = 0.003), and without care (P = 0.003).

4.5 DISCUSSION

In this chapter, I used a quantitative genetic framework to get a better understanding of the role of parental care in evolution. Phenotypes vary between environments, but how that variation is partitioned determines how rapidly that

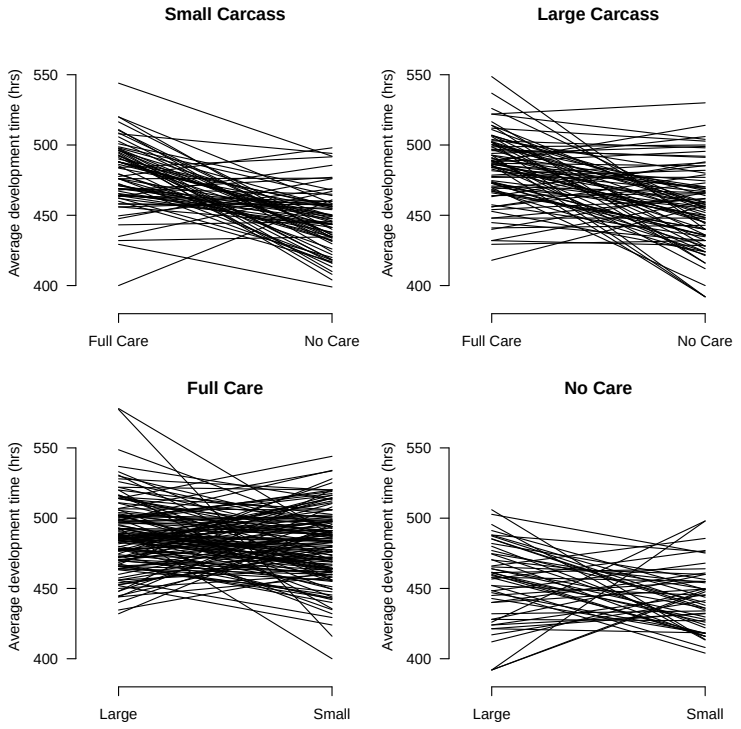


Figure 4.7 | Reactions norms of development time between all four environments. The top row of plots shows the reaction norm between the two parental care environments on either a Small carcass (left) or a Large carcass (right). The bottom row shows the reaction norm for each dam across carcass environments in Full Care (left) and No Care (right). There was significant dam × environment interaction across care environments on a small ($P < 0.001$) and a large carcass ($P < 0.001$), and across carcass environments with care ($P = 0.001$), and without care ($P < 0.001$).

trait evolves, and, indeed, if it can at all. In a full-sib/half-sib design, burying beetle larvae were reared in environments made up of a social component (Full Care or No Care) and a resource component (Small Carcass or Large Carcass), yielding four environments in total. I partitioned out the phenotypic variation to genetic, maternal, and residual causes to determine whether the absence of parental care unveils greater genetic variation upon which selection can act, or whether trait evolution can only evolve through maternal effects.

4.5.1 *Phenotypic effects of care and carcass*

I found there was a small effect of the interaction between care and carcass, where lighter, smaller, and faster developing larvae were produced on a small carcass without maternal care than on a large carcass without maternal care. When the mother was present, there was no difference in larval mass, development time,

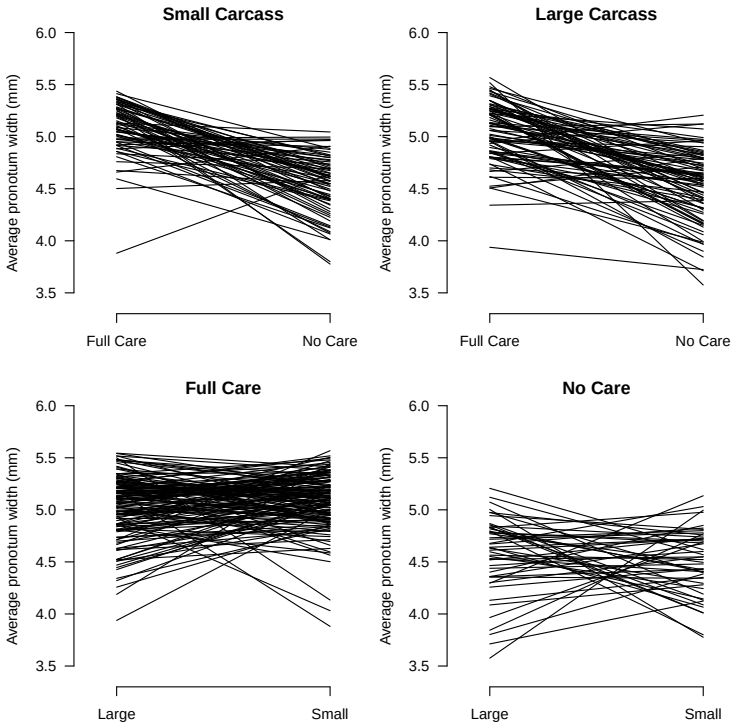


Figure 4.8 | Reactions norms of pronotum size between all four environments. The top row of plots shows the reaction norm between the two parental care environments on either a Small carcass (left) or a Large carcass (right). The bottom row shows the reaction norm for each dam across carcass environments in Full Care (left) and No Care (right). There was significant dam × environment interaction across care environments on a small (P = 0.002) and a large carcass (P = 0.046), and across carcass environments with care (P > 0.001), and without care (P = 0.008).

or adult pronotum width between carcass sizes (Figure 4.3, 4.4, and 4.5). This result aligns with my prediction and the literature. The presence of parental care increases the mass of larvae (Eggert, Reinking and Müller, 1998; Rauter and Moore, 2002), and also interacted with the mass of the carcass, a factor known to influence offspring phenotype (Andrews, Kruuk and Smiseth, 2016; Bartlett and Ashworth, 1988; Scott and Traniello, 1990; Smiseth and Moore, 2002a; Smiseth et al., 2014; Trumbo, 1991). As predicted, when parents were absent the large carcass environment yielded larvae that were heavier and larger than those reared on a small carcass, indicating a lack of constraint on offspring phenotype imposed by competition on a small resource. I did not find a similar pattern, however, when the mother was there to care for her offspring. Andrews, Kruuk and Smiseth (2016) found that with parental care, parents reared larger offspring on a larger carcass, which I did not find. The discrepancy between my result and that of Andrews, Kruuk and Smiseth (2016) could be explained by the larger carcass size class I used, which mirrored the large carcasses outlined in Chapter 3, whereas

the large carcasses used by Andrews, Kruuk and Smiseth (2016) match that of the medium carcass size. Interestingly, both large carcass ranges used are likely outside of the carrion niche used consistently by *N. vespilloides* in the wild (at least in the two Cambridgeshire populations outlined in Chapter 3).

4.5.2 *The effect of care and carcass on additive genetic variation*

While the mean phenotype did depend on the interactions between care and carcass, the levels of additive genetic variation did not increase in response to a change in the social environment, nor a change in the resource environment. In all traits across all environments, additive genetic variation was not different from zero, save larval mass in the Full Care Small Carcass environment (Table 4.2). The errors associated with these results are large, as they are across the other traits in the difference experimental environments. This suggests that the estimates are unlikely to accurately reflect the true heritability of larval mass. It is also strange that this result does not also hold for development time or pronotum width in the Full Care Small Carcass environment, given how strongly linked they are (Table 4.5). My results differ slightly with those of Rauter and Moore (2002), who find no heritability of larval size across care and no care environments, but significant additive genetic variation in development time (which they term as "in the pupal chamber") independent of parental care. Rauter and Moore (2002) used *N. pustulatus*, a different burying beetle species, whose interesting natural history may account for their different results. Firstly, *N. pustulatus* has evolved as a brood parasite (Trumbo, 1994, 1992), and secondly, *N. pustulatus* has evolved to utilise a unique host—snake eggs (Smith et al., 2007)—which may have changed effects on brood development and the role of parental care in governing development.

My results also do not fit with the consensus that a population's capacity to evolve is enhanced by a shift to a more stressful environment (Hoffmann and Merilä, 1999; Hoffmann and Parsons, 1991; Kawecki, Barton and Fry, 1997; Rowiński and Rogell, 2017). Removing parental care in *N. vespilloides* can be interpreted as a stressful environment where larvae grow slower and achieve a smaller mass at dispersal (Rauter and Moore, 2002). A smaller carcass, might be viewed in a similar way since offspring are smaller when they develop on one (Figure 4.3). I found no difference in additive genetic variation across either gradients of stress, which also does not support the idea that a more favourable environment increases additive genetic variance (Charmantier and Garant, 2005; Gebhardt-Henrich and Van Noordwijk, 1991). Nor did I find a difference between the two morphological traits (larval mass and adult pronotum size) or single life-history trait (development time) I measured. Morphological traits have higher

heritabilities than life-history traits in general (Postma, 2014), though life-history traits have greater genetic variation (Houle, 1992) due to their developmental instability (Price and Schluter, 1991), and so have greater evolutionary potential. Contrary to Rauter and Moore (2002) who found significant heritability of development time, independent of care, I found no such pattern between the morphological and life-history traits measured, presumably due to the inherent plasticity of these traits (Gavrilets and Scheiner, 1993).

Do parents buffer genetic variation from purifying selection and lead to its release if they are removed? My data suggest not, at least in the three traits I measured (Tables 4.2, 4.3 and 4.4). One explanation could be that strong selection has acted on the traits in the environments in which I measured them, and reduced genetic variation (Mousseau and Roff, 1987). Selection may have also favoured the evolution of phenotypic plasticity to cope with the uncertain environmental and social conditions, which may lead to unpredictable changes—including no change—in V_A across environments (Gavrilets and Scheiner, 1993). One criticism in investigating how changes in V_A may indicate cryptic genetic variation is that V_A is only measured in two environments. The environments in which cryptic genetic variation could be revealed may not be captured by such a dichotomy (McGuigan and Sgro, 2009). Experimental work has firstly focused on stressful environments where strong deviations from the norm are supposedly more likely to reveal cryptic genetic variation (e. g. stressful environments like temperature or nutritional extremes, Grill, Moore and Brodie, 1997; Imasheva et al., 1998). Experimenters have also focused on what may have been the environmental extremes by which a species has diverged (e. g. salinity gradients of three-spine stickleback, McGuigan et al., 2011). The larger the number of environments V_A is assessed in the better, as there is no indication what environmental change could reveal cryptic genetic variation (McGuigan and Sgro, 2009). I used four environments and did not find any differences, which may indicate parental care does not lead to the accumulation of genetic variation, at least in the three traits measured, or that the environment I chose was not the dimension of the species' ecology where cryptic variation would be released following the removal of parents.

Other work (Pilakouta et al., 2015; Snell-Rood et al., 2016) tantalisingly indicates that something is missing from our understanding of parental care as a buffer of selection. Both these studies used more direct, but less quantifiable, methods of (potentially) increasing deleterious mutations—inbreeding and radiation. Slightly deleterious mutations can be protecting from purifying selection by parental care, affording the opportunity for epistasis to arise between new mutations and open new adaptive trajectories that may have been closed (Covert et al., 2013; Gong and Bloom, 2014). A more promising possibility is to utilise

populations that have been evolving with and without parents (Schrader, Jarrett and Kilner, 2015b; Schrader et al., 2017) and expose them to a novel environment, like a change in temperature, or use them in a selection experiment. The prediction here would be that the population without the evolutionary history of parental care would, with the lack of genetic variation, fare less well in a novel environment, or respond less quickly to selection.

4.5.3 *The effect of care and carcass on maternal/permanent environment and residual variation*

While little phenotypic variation was explained by additive genetic variation, maternal/permanent environmental variation explained a considerable amount of phenotypic variation in all three traits in all four environments (Tables 4.2, 4.3 and 4.4). The maternal variation encompasses the direct genetic effects of the mother and permanent environment effects, so also includes maternal effects. Maternal effects have been shown to be larger under poor conditions when the parents are not there to care for their offspring (Gebhardt-Henrich and Van Noordwijk, 1991; Laugen et al., 2005; Merilä and Fry, 1998; Wilson et al., 2006). Maternal contributions through the environment, direct genetic effects or maternal effects more generally, govern the evolutionary potential of larval mass, development time and pronotum width. When comparing between Full Care Large Carcass and Full Care Small Carcass, I found that the maternal variation was greater on the large carcass. This means there is greater divergence in brood means from the population mean (where the brood is the group in Figure 4.1b), which suggests maternal effects shaping offspring phenotypes are likely larger on a large carcass.

When comparing between care environments, the variation partitioned into the dam component did not differ. This implies the effect of the dam persists when she is not there to care for her offspring. If the forces governing the final phenotype of the brood are solely determined by sibling interactions, the variation would be less partitioned into the dam component and more in the residual component. In fact, there is evidence to suggest that residual variance increases when the dam is present (Table 4.7); that is, parental care increases the variation in larval mass and pronotum width within a brood. This is contrary to what Rauter and Moore (2002) found, where variation in larval mass increased when parents were absent. *N. pustulatus* by virtue of a slightly diverged natural history with large clutches, may differ in the dynamics between parents and offspring, and between siblings. With large clutches and broods, more competitive interactions between siblings may have evolved as any cooperation among larvae is swamped by competition.

Mothers appear to influence how offspring interact with each other, beyond the effects they already impose. One could imagine a provisioning mother changing the extent of conflict between siblings, both in terms of competition but also by reducing potentially aggressive acts like sibling cannibalism (Wong, Lucas and Kölliker, 2014). For example, *Drosophila* larvae evolved a greater propensity to cannibalise other larvae, including their siblings (Vijendravarma, Narasimha and Kawecki, 2013), when they evolve under a nutritionally restricted diet; an environmental factor over which parents have a large influence. Alternatively, mothers exacerbate competition between offspring (Smiseth, Ward and Moore, 2007), which could account for the greater variation when parents cared for their offspring. If both parents and offspring contribute to a public good that benefits the whole family, the dynamics can shift when the parents are there compared with when they are not. In the burying beetles, this public good could be the production of exudates that influence the microbial community on the carcass (Arce, Smiseth and Rozen, 2013; Duarte et al., 2015), or the production of enzymes that aid breaking down the carcass for the benefit of all. Accessing the resources within the carcass is also a public good, where larvae and parents both contribute to creating an access point. Removing the parents changes the interactions between the offspring from competitive to cooperative at small brood sizes (Schrader, Jarrett and Kilner, 2015a). Variation exists in burying beetle families in the degree to which removing parental care changes the sibling dynamics. This is why I see considerable variation explained by the maternal environment, even in her absence, and it explains why the reaction norms in larval traits cross (Figures 4.6, 4.7 and 4.8). The significant maternal and residual variation estimates indicate that it is the combination of how parents and offspring interact, how siblings interact, and how parents mediate sibling interactions that holds the potential to change the evolution of the three traits I measured, even without additive genetic variation (Bijma and Wade, 2008; Wade, 1982, see Chapter 5).

4.5.4 *Dam × environment interactions*

Different strategies maximise fitness in different environments (Via and Lande, 1985). In this experiment, I found that different dams do best in different environments. This is because the reaction norms between environments were not flat, and because their rank order was not correlated between environments (i. e. the female that produced the largest offspring in one environment did not necessarily produce the largest in the alternative environment, Figures 4.6, 4.7 and 4.8). In variable environments, where parental care may be not guaranteed (see Chapter 6, Parker et al., 2015; Trumbo, 1992) and the size of the breeding resource cannot be predicted, phenotypic plasticity results from the accumula-

tion of maternal genes that are favoured in one environment but not another. In some cases, dams had larger offspring when they were prevented from caring for offspring. *N. vespilloides* is a facultative care-giver, and so it is not surprising that in some cases, despite the advantages maternal care provides, variation exists and offspring sometimes do better without care. Indeed, this variation is not just extrinsic (Trumbo, 1992; Wilson and Fudge, 1984), but also intrinsic (Chapter 6, Parker et al., 2015), so one can imagine that parents which are more likely to leave and abandon their parental duties have offspring that are better able to cope without care. The genetic variation in traits associated with surviving without parental care can be selected with experimental evolution studies (Schrader, Jarrett and Kilner, 2015b; Schrader et al., 2017). It would be interesting to replicate this experiment with populations that have been evolving without parental care, and ask whether the variation and dam × environment interactions are much weaker, as one would expect if only some trait combinations survive selection in this way.

Dam × environment interactions act to maintain phenotypic and potentially maternally-derived genetic variation within a population, which could have large consequences for subsequent adaptive evolution. The genes also derived from the mother interact with genes in the offspring, not necessarily only those that determine their phenotype directly, but those that contribute indirectly through feeding, for example. Intergenomic epistasis between maternal and offspring genes could also be a contributing factor in creating and maintaining dam × environment interactions (Linksvayer, 2007, 2006), where combinations of larval and parent genes are best fitted for certain environments. Parent and offspring are then expected to coevolve and become coadapted (Wade, 1998; Wolf and Brodie, 1998) which theoretically could speed up the pace of evolution, especially in traits without any additive genetic variance. The potential for genes to be adaptive in certain environments, whilst also being conditional on the genes in a social interactant, creates substantial genetic variation. This may facilitate adaptation to truly novel environments, which individuals may not have experienced before in their evolutionary history. Upon entering what Lande (2009) has termed an 'extraordinary' environment, the evolution of plasticity is rapidly favoured from a state of canalisation. The Large carcass in this experiment could be one such environment, as the probability of *N. vespilloides* managing to secure such a breeding resource in nature is very small considering the interspecific competition from larger *Nicrophorus* species (Chapter 3, Trumbo, 1990, 1992; Wilson and Fudge, 1984). Initial plasticity could provide variation sufficient enough to cope with even an extraordinary environment, through pleiotropic effects and intergenomic epistasis between parents and their offspring.

4.5.5 *Conclusion*

Parental care in *N. vespilloides* appears not to facilitate the accumulation of cryptic genetic variation in the three traits (larval mass, development time, and pronotum width) I measured. However, parents have the potential to facilitate evolutionary change through maternal effects and the maternal influence on offspring interactions. Parental care led to greater phenotypic variation in larval mass and adult body size within a brood. Phenotypic variation is also more driven by the dam in some environments, which suggest that maternal effects will have a greater influence in evolution when resources are plentiful. It is the significant maternal and residual variation across environments that indicates the potential for evolutionary change in body size and development time; a process that is reliant on the interactions between parents and offspring, offspring interactions, and how mothers mediate sibling interactions.

Part III

EVOLUTIONARY CONSEQUENCES OF PARENTS

5

COOPERATIVE INTERACTIONS WITHIN THE FAMILY ENHANCE THE CAPACITY FOR EVOLUTIONARY CHANGE IN BODY SIZE

This chapter has been published as:
Jarrett, B. J. M., 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

5.1 SUMMARY

Classical models of evolution seldom predict the rate at which populations evolve in the wild. One explanation is that the social environment affects how traits change in response to natural selection. Here I determine how social interactions between parents and offspring, and among larvae, influence the response to experimental selection on adult size. The experiment focuses on the burying beetle (*Nicrophorus vespilloides*), whose larvae develop within a carrion nest. Some broods self-feed on the carrion while others are also fed by their parents. I found that populations responded to selection for larger adults but only when parents cared for their offspring. I also found populations responded to selection for smaller adults, but only by removing parents and causing larval interactions to exert more influence on eventual adult size. Synthesising these results with previous studies, I suggest that cooperative social environments enhance the response to selection whereas excessive conflict prevents further directional response to selection.

5.2 INTRODUCTION

Predicting the rate at which populations can evolve and adapt in a rapidly changing world is a major challenge for evolutionary biology (Carroll et al., 2014). A key problem is to explain how rapidly traits change in response to selection. The Breeder's equation summarises classical genetic models of evolution by suggesting that the magnitude of evolutionary change in any given trait depends simply on the extent to which that trait contributes to fitness (the strength of selection), and the degree to which it is transmitted to the next generation by genetic variation (the trait's heritability, Falconer and Mackay, 1996; Lush, 1937).

Yet these two parameters are seldom sufficient to predict how evolution will proceed in the wild (McAdam and Boutin, 2004; Merilä, Sheldon and Kruuk, 2001). One suggestion is that this is because the social environment has an additional causal influence on the response to selection (Drown and Wade, 2014; Lande and Kirkpatrick, 1990; McGlothlin et al., 2010; Moore, Brodie and Wolf, 1997; Wolf, Brodie and Moore, 1999). An individual's social environment is derived from its behavioural interactions with conspecifics. Variation in the social environment can contribute to variation in an individual's phenotype, much as the abiotic environment does (Bourke, 2011; West et al., 2006). An important difference, though, is that there is genetic variation in the social environment. This means that the social environment can be inherited and can therefore change the response to selection of the traits that it induces (Drown and Wade, 2014; McGlothlin et al., 2010; Moore, Brodie and Wolf, 1997; Wolf, Brodie and Moore, 1999).

Specifically, mathematical analyses show that when the effects of the social environment on trait expression (typically denoted Ψ) is a large and positive effect, it increases a trait's response to selection and accelerates evolutionary change (Bailey and Zuk, 2012; Bleakley and Brodie, 2009; Chenoweth, Rundle and Blows, 2010). But, if the effect of the social environment is negative, it prevents any response in the trait to selection and impedes evolutionary change (Bijma, 2014; Bijma and Wade, 2008; Drown and Wade, 2014; Hadfield, 2012; Kirkpatrick and Lande, 1989; McGlothlin et al., 2010; Moore, Brodie and Wolf, 1997; Wade et al., 2010; Wolf, Brodie and Moore, 1999). Previous experiments with domesticated species have supported that latter prediction by showing that competitive interactions can prevent selection for traits of greater economic value to farmers, such as increased body size (Bergsma et al., 2008; Bijma, 2014; Hadfield, 2012; Kirkpatrick and Lande, 1989; Wade et al., 2010). However, it is unclear whether the social environment can ever causally accelerate trait evolution in animal populations. Nevertheless, theoretical work (Drown and Wade, 2014; McGlothlin et al., 2010; Moore, Brodie and Wolf, 1997; Wolf, Brodie and Moore, 1999) and correlational analyses of the outcome of natural selection using large pedigreed datasets collected from wild animals, both suggest it is likely (Wilson et al., 2011).

I tested whether the social environment within the family can promote the evolution of body size in burying beetles (*Nicrophorus vespilloides*) using experiments on wild-caught individuals. This species exhibits facultative biparental care (Chapters 2 and 3, Capodeanu-Nägler et al., 2016; Eggert and Müller, 1997), which makes it ideal for experimental manipulations of the social environment (Chapter 4, Schrader, Jarrett and Kilner, 2015b). I focused on the evolution of adult size for four reasons. First, parental care and the evolution of body size

across the *Nicrophorus* genus is highly correlated (Chapter 3). Second, body size is strongly associated with fitness in *N. vespilloides*, the species of this experiment (Scott, 1998b). Competition for the carrion breeding resource can be intense, and larger beetles are more likely to win fights for ownership of the carcass (Otronen, 1988). Third, adult size is known from previous work to vary with aspects of the family social environment that larvae experience during development, including cooperative social interactions with siblings at low densities (Schrader, Jarrett and Kilner, 2015a) and cooperative interactions with parents (Eggert and Müller, 1997). And fourth, I found that the heritability of adult size is very low in both social environments where parents are present (Full Care) and where they are absent (No Care) (Chapter 4). This gives the opportunity to distinguish the effect of the social environment from effects due to the heritability of body size on the way body size responds to selection. This is because body size should exhibit negligible change as a function of its heritability.

5.3 METHODS

5.3.1 *Experimental design*

To test whether the social environment causally influences the response to selection, I carried out an artificial selection experiment on eight laboratory populations. Importantly, I varied the social environment among the populations so that I could analyse its causal influence on the response to selection: half the populations experienced Full Care during development (N = 4 populations), the other half had No Care (N = 4 populations). I then exposed half of the populations within each Care environment to selection for increased adult body size (Large), while the remaining populations experienced selection for decreased adult body size (Small, Figure 5.1). Thus I had four types of experimental populations, each replicated twice: Full Care Large, Full Care Small, No Care Large, and No Care Small. I selected on body size for seven generations, generating over 25,000 beetles.

5.3.2 *Artificial selection experimental populations*

All of the individuals used in the selection experiment belonged to a captive colony established at the University of Cambridge in 2013 from wild-caught adults collected under license from local field sites at Byron's Pool and Wicken Fen in Cambridgeshire, UK. Full details can be found in Schrader, Jarrett and Kilner (2015b). In short, wild-caught individuals were bred within and between

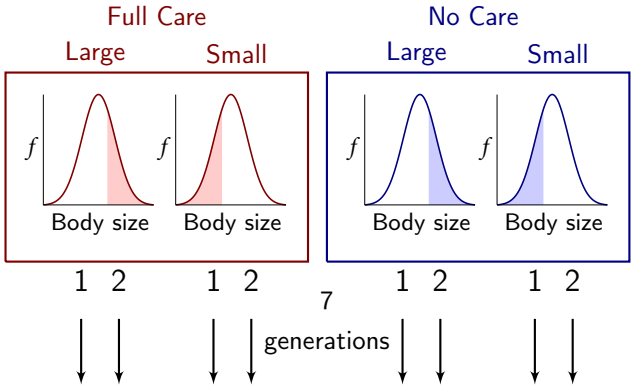


Figure 5.1 | A schematic diagram detailing the artificial selection experiment. Within the two Care treatments, Full Care (red) and No Care (blue), there are two selection regimes, selection for Large adult size and selection for Small adult size, shown by the shaded section of the normal distribution. I continued this selection for seven generations with two replicate populations per treatment.

sites to produce a large, genetically diverse population, which was maintained in the laboratory for four generations.

From this genetically diverse founding population, I started eight populations consisting of four treatments with two replicates per treatment, randomly allocating individuals to treatments. I had two treatments, Parental Care and Selection, resulting in a 2 × 2 factorial experiment (Figure 5.1). Parental Care was manipulated by either leaving or removing both parents 53 hours after pairing, after carcass preparation and egg laying were complete (Boncoraglio and Kilner, 2012), resulting in a Full Care treatment and a No Care treatment, respectively (Chapter 2). I then imposed two selection regimes on the Full Care and No Care populations: Large and Small (Figure 5.1). I selected the largest third of the population with the Large regime, and the smallest third of the population under the Small regime. Selection was imposed at the population level and not at the family level. Once the population had been selected, individuals were paired randomly, ensuring cousins and siblings did not breed. All beetles were maintained in the conditions outlined in Chapter 2. Each population was maintained with at least 25 families per generation, by breeding 40 pairs of beetles for the Full Care populations and 60 pairs for the No Care populations. Extra pairs were bred in the No Care populations to ensure enough successful families: failure rates are high when initially removing parents (Schrader et al., 2017). When it became impossible to sustain populations of this size, the experiment ceased.

At eclosion, members of the same sex from each family were temporarily housed in a box together and anaesthetised with CO_2. Once anaesthetised, each individual was photographed and the body size measured, using the same meth-

ods described in Chapter 3 with a custom MATLAB script. Each individual was given a unique ID that I used to identify individuals that were retained to breed for the subsequent generation.

To estimate the potential for evolutionary change in body size in each population, I calculated the realised heritability of body size, as the slope of the regression of the cumulative response to selection against the cumulative strength of selection (Lynch and Walsh, 1998). I included experiment population replicate as a two-level fixed effect and found it did not significantly explain any variation (F_{40} = 2.08, P = 0.10). I therefore pooled both replicates for subsequent analyses. After the global model, I used pairwise comparisons to compare measures of realised heritability across treatments. Post-hoc pairwise comparisons were adjusted for multiple testing using false discovery rate (Benjamini and Hochberg, 1995).

5.3.3 *The effects of the social environment on adult size*

The social environment that larvae experience during development influences the size larvae attain by the time they disperse from the carcass, and this, in turn, is strongly correlated with adult size (Lock, Smiseth and Moore, 2004). Three factors contribute to this social environment: clutch size, brood size, and the presence (or absence) or parental care during larval development (Schrader, Jarrett and Kilner, 2015a). To understand how these difference elements of the social environment might have caused the outcome of the selection experiment, I investigated how clutch size and brood size are related to adult size.

To assess the effect of female size on clutch size, I analysed data from Schrader et al. (2016) where the authors manipulated female size experimentally and counted the total clutch size for a breeding attempted after 53 hours, when egg laying had ceased (Boncoraglio and Kilner, 2012). Brood size data were taken from a stock population maintained in the laboratory under the same conditions as the Full Care populations, and assayed when the artificial selection populations were in generation five. Brood size was measured at the point of larval dispersal away from the carcass. Both clutch size and brood size were analysed with a Poisson distribution and a log link function with female size and carcass mass fitted as covariates.

The next step was to assess the role of adult size on clutch size and brood size and relate these measures to the results of the artificial selection experiment. If the outcome of the selection experiment is attributable to different elements of the social environment, then I predict I should see divergence in clutch size and brood size at dispersal among the different experimental populations.

To do this, I estimated clutch size in all experimental populations at generation five by counting the number of eggs visible on the bottom of the breeding box. This measure is strongly correlated with total clutch size (Schrader et al., 2016). I analysed clutch size using a generalised linear model with a Poisson error structure and log link function and included carcass size as a covariate whilst testing for the interaction between selection regime and care treatment. I used this approach, too, for brood size at dispersal at generation seven of the experiment.

From the proposed mechanism behind the response to selection, I predicted that the slope of offspring size regressed against dam size would differ among the experimental treatments. Specifically, I predicted that the slope would be positive for the Full Care Large and No Care Small lines, because these were the lines in which I observed phenotypic change. I predicted that the slope would be negative in the No Care Large and Full Care Small lines, as there was no phenotypic change in these lines. I took all the data from all the lines and combined both replicates per treatment for the seven generations of the experiment.

I ran a linear mixed effects model coding the three-way interactions of Care treatment against selection regime and dam pronotum width. I also included carcass size and generation as covariates. Dam ID was fit as a random term. Significance was determined by removing the three-way interactions from the model and comparing the output with the full model. The slopes for each experimental treatment were obtained in the same way but with the appropriate subset of the data for each experimental treatment. I used `R` (R Development Core Team, 2016) and the package `lme4` (Bates et al., 2015) for all analyses.

5.4 RESULTS

5.4.1 *The realised heritability*

The realised heritability of body size varied among the four experimental treatments ($F_{3,44} = 6.87, P < 0.001, N = 48$, Figure 5.2). Furthermore, the realised heritability of body size was relatively high, and significantly different from zero, for the Full Care Large treatment (0.09 ± 0.02), where body size increased across the generations, and for the No Care Small treatment (0.11 ± 0.03), where mean body size correspondingly decreased. For these two treatments I therefore conclude that the social environment during development enhanced the capacity for evolutionary change in adult body size, and to a similar degree whether selection was for increased or decreased body size.

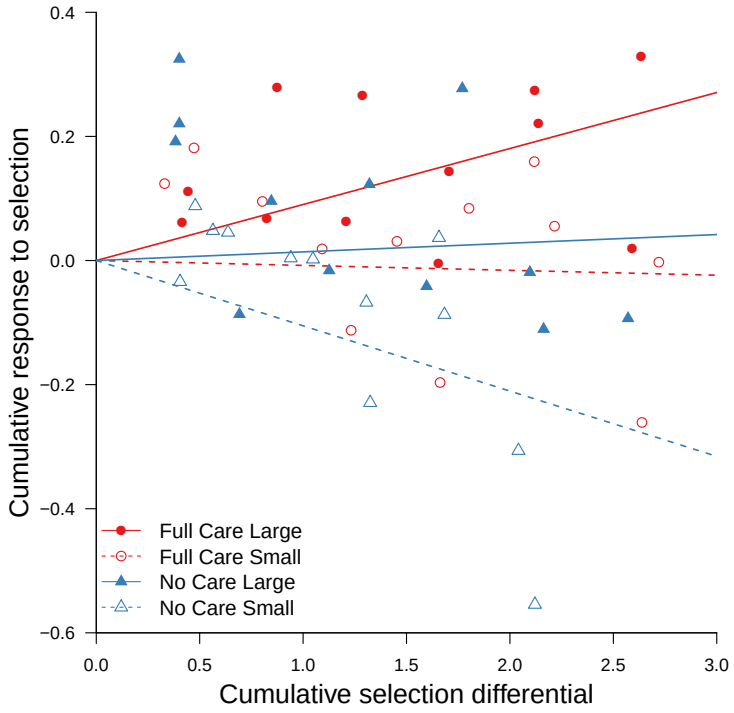


Figure 5.2 | The realised heritability of body size in each of the four treatments. The cumulative selection differential is the difference between the population mean and the mean of the retained subset of the population. This is summed together across the generations. The cumulative response to selection is the difference between the mean of the population and the mean of the population in the subsequent generation, which is also summed together. The slopes of this regression, forced through the intercept, is the realised heritability, and for each treatment are: Full Care Large, 0.09 ± 0.02; Full Care Small, −0.01 ± 0.02; No Care Large. 0.01 ± 0.03; No Care Small, −0.11 ± 0.03.

By contrast, in the Full Care Small and No Care Large treatments, the realised heritability of adult body size was not significantly different from zero (Full Care Small: −0.01 ± 0.02; No Care Large: 0.01 ± 0.03). Mean adult body size did not change over the course of the selection experiment for populations from either of these treatments (Figure 5.2).

The Full Care Large and Full Care Small treatments significantly differed from one another in their realised heritability estimate ($F_{22} = 9.90$, $P_{adj} = 0.015$), as did the Full Care Large and No Care Small ($F_{22} = 26.44$, $P_{adj} = 0.006$). There was marginal support for a difference in realised heritability between the Full Care Large and No Care Large ($F_{22} = 3.95$, $P_{adj} = 0.072$). Realised heritability in the No Care Small differed significantly from that in the Full Care Small ($F_{22} = 5.92$, $P_{adj} = 0.03$) and the No Care Large populations ($F_{22} = 6.36$, $P_{adj} = 0.03$). The Full Care Small and No Care Large did not differ from one another in their

realised heritability (F_{22} = 0.30, P_{adj} = 0.59). Realised heritability estimates for each population are in Table 5.1.

Table 5.1 | The realised heritabilities for each of the eight populations.

Population	Realised heritability	Standard error	*t* value
Full Care Large 1	0.05	0.03	1.929
Full Care Large 2	0.13	0.02	5.401
Full Care Small 1	-0.04	0.04	-1.030
Full Care Small 2	0.02	0.02	1.140
No Care Large 1	0.07	0.06	1.047
No Care Large 2	-0.02	0.02	-0.917
No Care Small 1	-0.13	0.05	-2.617
No Care Small 2	-0.08	0.04	-1.771

5.4.2 *Clutch size and brood size*

I used independent populations to ask how female size influenced both clutch size and brood size. I found that clutch size increased with female size, accounting for carcass mass (t = 3.63, P = 0.001, Schrader et al., 2016), whereas brood size at dispersal decreased with female size (t = −2.06, P = 0.04, Figure 5.3), most likely through the act of filial cannibalism (Bartlett, 1987).

Based on the results from Figure 5.3, I predicted that the clutch size should be greater in populations where adults are selected to be larger (Full Care Large and No Care Large) than in populations where adults are selected to be small (Full Care Small and No Care Small). That is indeed what I found (Figure 5.4). In the fifth generation of the selection experiment, clutch size was greater in the Large selected lines than in the Small selected lines (z = −7.53, $P < 0.001$), independent of the parental care treatment (z = 1.32, P = 0.19). There was no interaction between selection regime and parental care on clutch size (z = −0.38, P = 0.70).

Again, based on the results from Figure 5.3, I predicted that brood size would be both affected by the selection regime and the care treatment, with the Full Care Large populations having a smaller brood size, as parents are present to eat offspring. Since there is no possibility of filial cannibalism in the No Care populations, the brood sizes of these populations should vary in the same way as clutch size. As predicted, I found that there was a significant interaction between selection regime and care treatment in the brood size of the experimental populations at generation seven (z = −4.89, $P < 0.001$, Figure 5.5). Full Care Large populations had fewer offspring at dispersal than the Full Care Small

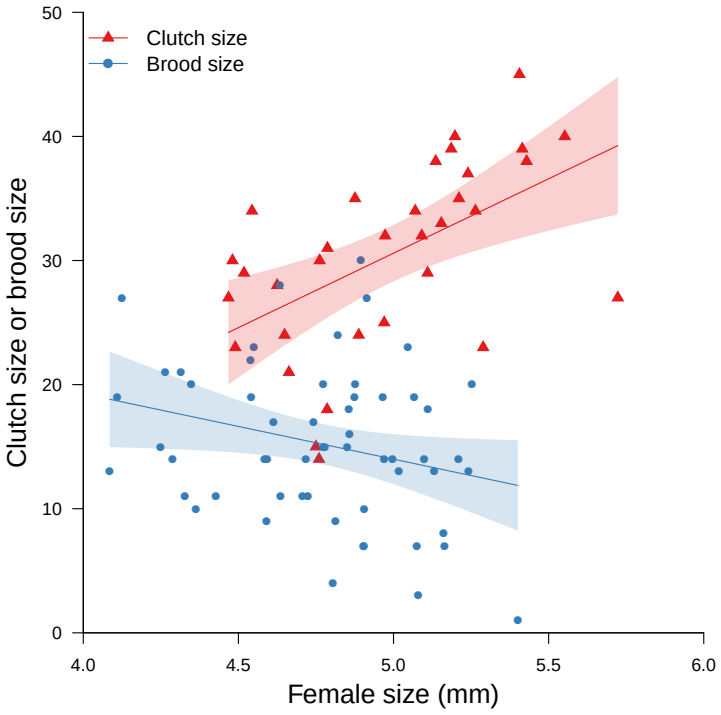


Figure 5.3 | The relationship between female size and a) clutch size (in red triangles); and b) brood size (in blue circles). Clutch size (red line, N = 33) increases with female size. Data were taken from Schrader et al. (2016). Brood size (blue line, N = 55), decreases with female size. Female size refers to pronotum width. Each datapoint corresponds to a different female.

populations, whereas No Care Large populations had more offspring at dispersal than No Care Small populations.

I tested how dam size contributed to the differential responses to selection found in the experimental populations, by comparing the slope of the regression between dam size and progeny size across the different treatments. Figure 5.6 outlines the likely mechanism behind the response to selection, and so predicts that the regression of dam size against progeny size should be positive in the Full Care Large and No Care Small lines, and negative in the No Care Large and Full Care Small lines. I found that the correlations differed significantly among treatments ($\chi^2_1 = 4.13, P = 0.042, N = 15,484$). The slopes were positive in the Full Care Large (0.13 ± 0.09) and No Care Small treatments (0.09 ± 0.09). However, although it was negative in the Full Care Small treatment (−0.06±0.06), as predicted, it was positive in the No Care Large treatment (0.12 ± 0.1), which I did not predict.

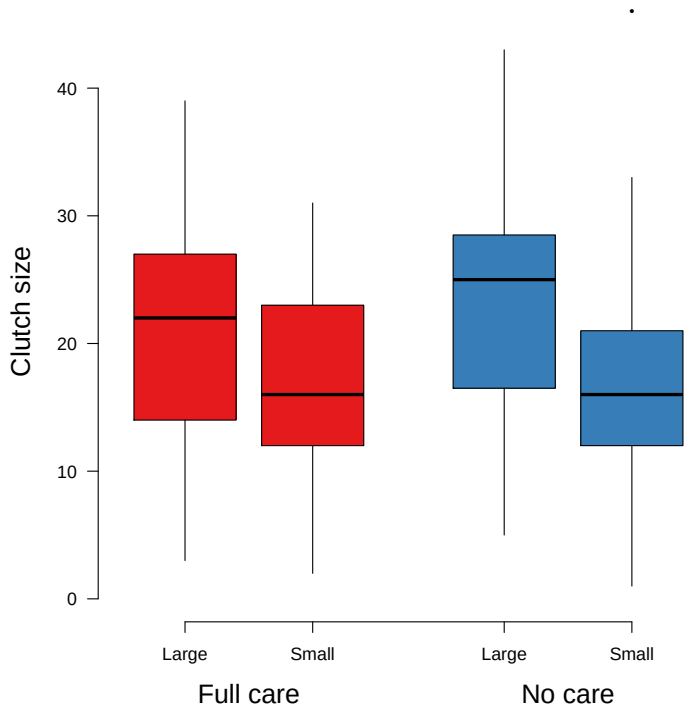


Figure 5.4 | Clutch size at generation five in the four different experimental treatments in the selection experiment: Full Care Large (N = 38) and Full Care Small (N = 39) in red; No Care Large (N = 51) and No Care Small (N = 44) in blue. Both replicates per treatment are combined. Box plots show median and interquartile ranges.

5.5 DISCUSSION

Here, I determined whether the social interactions within families determine the evolutionary trajectory of populations. Specifically, I found that cooperative interactions were necessary for a positive response to selection. I identified three elements of the social environment that interacted to determine the response to artificial selection on body size (Figure 5.6): clutch size, brood size, and the trade-off between larval size and brood size. Larval mass is a strong determinant of adult size (Lock, Smiseth and Moore, 2004), and is partly determined by the number of eggs laid by the female which dictates the number of competing larvae on the carcass. It is not the sole determinant of brood size on the carcass, however. Larger females lay a larger clutch (Schrader et al., 2016), but have fewer surviving larvae that disperse from the carcass (Figure 5.3), presumably due to a greater incidence of filial cannibalism (Bartlett, 1987), which can only occur when the parent is present post-hatching. Brood size is therefore different from clutch size, a difference that is contingent on the social environment, and is therefore the second social element influencing larval mass. The third factor is the presence or

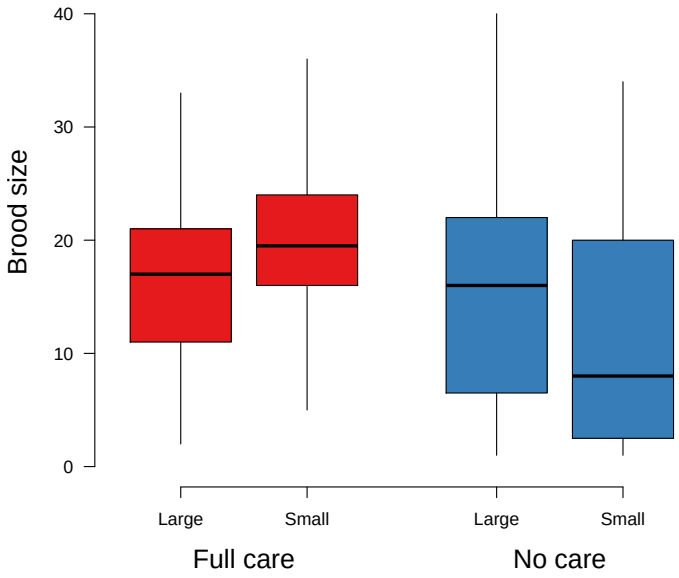


Figure 5.5 | Brood size at larval dispersal in the four different experimental treatments in the selection experiment: Full Care Large (N = 54) and Full Care Small (N = 52) in red; No Care Large (N = 47) and No Care Small (N = 15) in blue. Both replicates per treatment were combined. Box plots show the median and interquartile ranges of the data.

absence of parental care post-hatching. This is important because it influences the relationship between brood size and larval size at dispersal, especially for broods of 10 or fewer larvae (Schrader, Jarrett and Kilner, 2015a). When parents are present, and there are only a few larvae on the carcass, each larva consumes more carrion and are fed by their parents, and are larger at dispersal (Schrader, Jarrett and Kilner, 2015a). When parents are absent, however, each larva typically attains only a low mass by the time it disperses to pupate, because larvae seemingly help each other to colonise and consume the carcass (Schrader, Jarrett and Kilner, 2015b). Thus, larvae in small broods cannot attain a large mass at dispersal when parents are absent, but they can when parents are present.

I suggest that selection on these three elements of the social environment combined to cause correlated change in body size in the Full Care Large lines and the No Care Small lines. In the Full Care Large treatment (Figure 5.6a), I selected for larger adults. They produced larger clutches (Figure 5.4), but produced fewer (Figure 5.5) and therefore larger dispersing larvae (presumably due to the greater levels of filial cannibalism). They matured into larger adults themselves, reinforcing the feedback loop across generations. Likewise, in the No Care Small

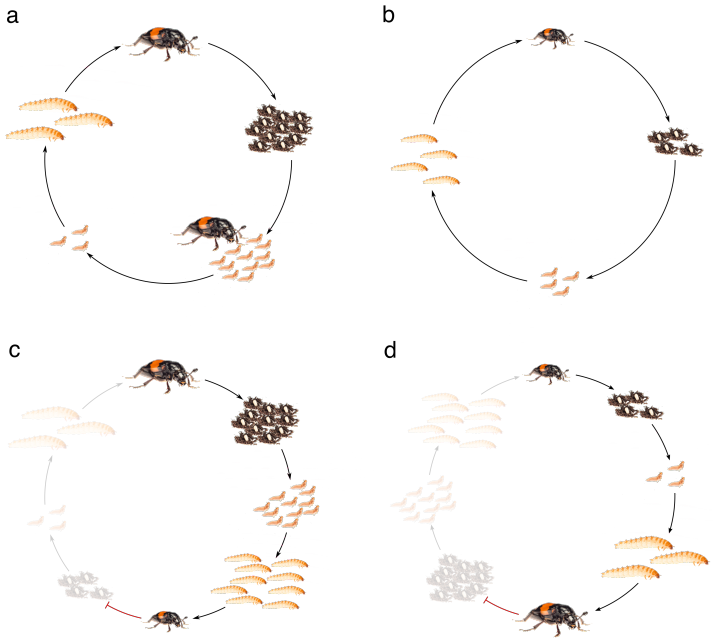


Figure 5.6 | The effect of the social environment on the response to selection, in each of the experimental treatments. **a, b** The social environment enhances the capacity for evolutionary change. **c, d** The social environment could prevent evolutionary change. **a** Full Care Large: large beetles lay many eggs, but are more likely to cannibalise larvae and therefore have relatively small broods that yield large larvae, which mature into large adults. **b** No Care Small: small beetles lay fewer eggs, which yield a small brood of small larvae that mature into small adults. **c** No Care Large: large beetles lay many eggs, which yield a larger brood of small larvae that mature into small adults and are selected out of the experimental population. **d** Full Care Small: small beetles lay fewer eggs which yield a small brood of large larvae that mature into large adults and are selected out of the population.

treatment (Figure 5.6b) I selected for smaller adults which laid a smaller clutch (Figure 5.4). Since these broods were reared without parents, the resulting small broods (Figure 5.5) yielded smaller larvae, which matured into small adults. In each treatment, we effectively selected a social environment on the carcass that induced the production of more individuals with either a larger (Full Care Large) or smaller (No Care Small) body size. Furthermore, these selected individuals then produced a similar social environment for their offspring. This explains why these lines responded to selection on body size, despite the very low heritability of body size.

I observed little change in body size in the other experimental populations (No Care Large and Full Care Small). This was predicted by the classical estimates of heritability (Chapter 4), but may also be attributed to effects of the social environment, which could have cancelled out the effects of selection at each

generation (Falconer, 1965). For example, in the No Care Large treatment (Figure 5.6c), selection for larger adults yielded smaller individuals in the next generation. The large adults laid a large clutch (Figure 5.3), but with no parents present after hatching to cannibalise offspring, these large clutches (Figure 5.4) yielded large broods (Figure 5.5) of small larvae, which matured into small adults that were not selected for the next generation. Similarly, in the Full Care Small treatment (Figure 5.6d) selection for smaller adults yielded larger adults in the following generation which were not selected. The small adults laid a small clutch (Figures 5.3, 5.4), which in turn yielded a small brood (Figure 5.5) of large larvae that matured into large adults.

While the data supports the above negative feedback loops for the No Care Large and Full Care Small treatments, the parent–offspring regression slopes support the idea that the lack of response is mainly determined by the extremely low classical estimate of the heritability of body size. I therefore find no clear evidence to conclude that the social environment within the family prevented any evolutionary change in these two treatments. The positive slopes of the Full Care Large and No Care Small treatments do however support the idea that social interactions within the family enhanced the response to selection. Specifically, I have shown that parental care is required to promote a rapid evolutionary increase in body size in *N. vespilloides*; a result that is consistent with the comparative analysis in Chapter 3 (Figure 3.8). Species that are completely dependent on parental care tend to be larger.

Setting our results alongside previous work on other species suggests that in general, the way in which the social environment influences a trait's response to selection depends on whether it is associated with social interactions that are cooperative or that promote excessive conflict (see West et al., 2006, for formal definitions of these terms). For example, previous studies have shown that selection for increased size or productivity in pigs and poultry also selects for increased aggression. Increased aggression reduces fitness so much that any effects of selection on size cannot be transmitted to the next generation and this prevents evolutionary change (Bergsma et al., 2008; Wade et al., 2010). This suggests that traits associated with social environments which induce high levels of conflict could have limited capacity for further directional evolutionary change. Previous work has also demonstrated that, under these conditions, the only way in which increased productivity or size can be artificially selected is by imposing multilevel, group or kin selection (Bijma and Wade, 2008; Wade et al., 2010). That is, a response to selection can be restored only when an explicitly cooperative social environment is artificially created at the same time (Queller and Strassmann, 2009).

Our experiment provides more direct evidence that cooperative interactions enhance the response to selection, and can do so even when selection acts on individuals. In the Full Care Large treatment, selection for increased body size was possible because parents helped small broods of larvae to attain a large size at dispersal (Schrader, Jarrett and Kilner, 2015a). In the No Care Small treatment, selection for smaller individuals decreased brood size, and smaller broods resulted in smaller larvae. This result can be explained by our previous finding that larval cooperation is key to larval success when parents are removed (Schrader, Jarrett and Kilner, 2015a). Presumably, with fewer siblings to help penetrate and feed upon the carcass, individual larvae in small broods were able to attain only a low mass by the time they dispersed from the carcass. Reducing the number of cooperating larvae thus reduced larval mass. In these two different ways, cooperative interactions reinforced the response to selection in our experiment by magnifying changes in body size across the generations, causing increases and decreases in body size of a similar magnitude. Cooperative interactions within the family therefore enhanced the capacity for evolutionary change.

Our general conclusion is that the response to selection is likely to be reduced when trait expression is associated with excessive conflict, but enhanced for traits whose expression is associated with more cooperative social environments. Proper characterisation of the social environment in which traits are expressed is therefore important not only for understanding a trait's current adaptive value (West et al., 2006) but also for predicting its future capacity to evolve and adapt.

Figure 5.7 | A cartoon depicting me presenting the results of Chapter 5 during a conference, kindly drawn by Matt Golding.

6

ADAPTIVE EVOLUTION OF MORPHOLOGICAL PLASTICITY IN RESPONSE TO A PREDICTABLE SOCIAL ENVIRONMENT

6.1 SUMMARY

Here I describe direct observations and experiments on burying beetles (*Nicrophorus vespilloides*), which focus on the scaling relationship between larval mandibles and larval body mass. The mandible allometry is flat in wild populations and associated with unpredictable levels of parental care during development. By experimentally evolving populations under different regimes of care, I show that the predictable absence of parental care imposes directional selection on small larvae to maintain disproportionately large mandibles. When parents are present, selection on the mandible allometry is weakened and small larvae have smaller mandibles. In further experiments, I show that the evolution of the mandible allometry is adaptive for the levels of care that larvae receive in each experimental population.

6.2 INTRODUCTION

How new adaptations arise from standing genetic variation is a longstanding question within evolutionary biology. Recent work suggests that changes in how sensitive—or plastic—a trait is to environmental cues could be key to driving adaptive phenotypic change (Nijhout and McKenna, 2017; Pfennig et al., 2010; Pigliucci, Murren and Schlichting, 2006; Suzuki and Nijhout, 2006; West-Eberhard, 2003). However, empirical analyses of this hypothesis have focused almost exclusively on the consequences of an evolutionary loss of sensitivity to environmental cues, which occurs when phenotypically plastic traits become genetically assimilated (reviewed in Levis and Pfennig, 2016). Here I determine whether adaptive morphological evolution can also result from an evolutionary gain in sensitivity to environmental cues.

I tested this idea by focusing on morphology, specifically the scaling relationship between trait size and body size (Huxley, 1932). In this context, the environment is the body size of the individual bearing the trait of interest (Emlen and Nijhout, 2000). The environmental sensitivity of a morphological trait can be quantified by measuring how it scales with body size among individuals at the identical stage of development (the trait's 'static allometry'; Emlen and

Nijhout, 2000). Static allometry is defined as $y = \alpha x^{\beta}$, where y is the size of the trait of interest, x is body size, α is the allometric intercept, and β is the allometric scaling parameter. Taking the natural logarithm of the trait size and body size yields a linear relationship, $\log(y) = \log(\alpha) + \beta \log(x)$, where $\log(\alpha)$ is the intercept and β the slope of the line. Morphological traits in the same dimension as body size are isometric (geometrically similar) when $\beta = 1$; that is, they scale proportionally to body size (Gould, 1966; Huxley, 1932; Shingleton and Frankino, 2013). A positive allometry is defined as $\beta > 1$, so that trait size increases disproportionately as organisms get larger; conversely, a negative allometry arises when $\beta < 1$. Here, smaller individuals have a disproportionately large trait relative to their body size.

Some traits are relatively invariant to body size ($\beta \ll 1$), like insect genitalia, which are the same size irrespective of the body size of the individual (Eberhard, 2009; Emlen et al., 2012; House and Simmons, 2007; Voje, 2016). Recent work has identified some of the genes and epigenetic mechanisms that cause traits to be sensitive to nutritional cues about body size (e.g. Emlen et al., 2012; Mirth, Frankino and Shingleton, 2016; Ozawa et al., 2016; Tang et al., 2011). Yet little is known about the nature of selection that acts on these genes, which ultimately determine scaling relationships (Pélabon et al., 2014). For example, the selective conditions that favour morphology that is independent of body size in wild populations are unclear (Eberhard, Rodriguez and Polihronakis, 2009; Pélabon et al., 2014; Stillwell et al., 2016), and the evolvability of static allometries is generally thought to constrain morphological evolution over short time scales, while it is unknown over longer time spans (Labonte et al., 2016; Pélabon et al., 2014; Stillwell et al., 2016; Voje et al., 2013).

Paradoxically, one way to investigate the evolution of morphological invariance to body size is to look at cases where morphological diversity is large. Some of the best understood examples of morphological evolution are the direct result of selection acting from the social environment—sexually selected traits (Arnqvist and Rowe, 2002; Eberhard, 1985; Emlen, 2008; Gould, 1973). Sexual selection results in traits that are both positively allometric, like antlers and horns (Emlen, 2008; Gould, 1973), and negatively allometric, like genitalia (Eberhard, 2009; Eberhard et al., 1998). Social selection, of which sexual selection is one aspect, can cause the fitness of social partners to covary, which creates feedback loops in which one individual influences the phenotype of its social partner and reciprocally induces a new phenotype in the first individual (McGlothlin et al., 2010; Moore, Brodie and Wolf, 1997; Trubenová and Hager, 2014; West-Eberhard, 1983; Wolf, Brodie and Moore, 1999).

The evolution of morphology has not been explicitly investigated in social environments outside the realms of sexual selection. Here, I investigate morpho-

logical evolution in the context of the social life of animal families. I describe observations and experiments on burying beetles (*Nicrophorus vespilloides*, Figure 6.1), which determine the adaptive value of the static allometry of larval mandibles, and the evolvability of this scaling relationship when selection changes. Burying beetles commonly exhibit elaborate biparental care, centred around the carcass of a small dead vertebrate. Parents convert the carcass into an edible nest for their larvae by removing the fur or feathers, covering the flesh in antimicrobial exudates, rolling it into a ball and burying it in a shallow grave (De Gasperin et al., 2016; Pukowski, 1933; Scott, 1998b). The larvae hatch from eggs laid nearby in the soil and crawl to the carcass. Parents assist the newly hatched larvae in penetrating the carcass and colonising it, by biting small incisions in the flesh. Once the larvae have taken up residence upon the carcass, parents may stay to defend them, to feed them via oral trophallaxis, and to tend to the carcass (Smiseth and Moore, 2002b). Larvae also feed themselves from the carrion and can survive without any post-hatching care at all (Smiseth, Darwell and Moore, 2003). I focus on mandible morphology as mandibles are the tools offspring use both for gaining access to the carcass without parental help (Benowitz et al., 2017), and because they have a role in larval self-feeding, both behaviours of which are significantly influenced by the presence or absence of post-hatching parental care.

Variation in the duration of parental attendance at the carcass, and in the extent to which the carcass is prepared prior to larval hatching, results in considerable uncertainty about the extent of parental assistance larvae will receive. Many factors influence the duration of parental care in burying beetles, like carcass size (Scott and Traniello, 1990; Trumbo, 1991), stage of larval development (Trumbo, 1991), interspecific interactions (De Gasperin, Duarte and Kilner, 2015), and seasonality (Meierhofer, Schwarz and Müller, 1999). Trumbo (1991) found that at exhumation of carcasses in the wild, both parents were absent in almost 10% of cases. Hence, I initially quantified the extent of parental care in wild populations in Cambridgeshire, including when both parents leave the brood, and the timing of the incision into the carcass made by the parents.

As with previous studies in both the field (Trumbo, 1991) and the laboratory (Parker et al., 2015), I predicted I would find considerable variation in the extent of parental care wild individuals would provide to their offspring. Variation in the social environment exerts considerable fluctuating selection on offspring, which I predicted would result in reduced variation in mandible size (Pélabon et al., 2010), and a negative allometry, as access to the carcass and self-feeding are crucial for larval fitness (Eggert, Reinking and Müller, 1998). I then used experimental evolution to test whether the wild mandible allometry is an adaptation to unpredictable levels of parental care by exposing populations to two different,

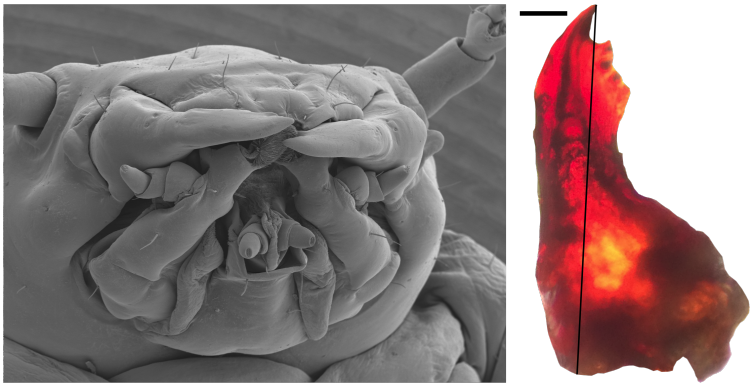


Figure 6.1 | An electron microscope image of an *N. vespilloides* third instar larvae (left) which displays the the larval mandibles. Electron microscopy image taken by Claudia Grossman. On the right is a dissected and set third instar larval mandible. The black line indicates the length of the mandible. The scale bar equals 90 µm.

but consistent, levels of post-hatching care. These were Full Care, where parents could not leave the box, and No Care, where parents were removed prior to larval hatching—i. e. the two extremes of variation in the social environment seen naturally. I predicted that the mandible allometry in the Full Care populations would evolve towards a more positive allometry (where β approaches 1) as parents are always present to care for their offspring, whereas the No Care populations would stay in stasis and maintain the flat allometry ($\beta = 0$) as parents are never present to care for their offspring. I finally tested the function of the evolved mandible allometry with two experiments, where I mimicked the action of the parents and made the incision into the carcass myself. If mandibles are crucial for larval survival and fitness, the number of Full Care larvae should increase when an artificial cut is made into a carcass.

6.3 METHODS

6.3.1 *Variation in parental departure*

To measure the variation of wild-caught individuals, I caught beetles from two natural populations (Gamlingay and Waresley Woods, see Chapter 3) in Cambridgeshire and kept them under identical conditions for one week in order to standardise conditions before the breeding. Individuals were randomly paired up within their respective population, while ensuring those from the same trap did not breed.

I placed the individuals into a large breeding box (28.5 × 13.5 × 12 cm) that had been divided into two sections, one twice as the large as the other. The divider had a hole cut into it, with a tube and cloth tunnel. The larger section was for

the breeding attempt where the parents would be provided with a carcass. The smaller section was for the parents to exit into, with the cloth tunnel used to prevent re-entry into the breeding chamber (De Gasperin, Duarte and Kilner, 2015). The wild pairs were provided with a recently defrosted mouse carcass (8–12 g) to initiate breeding. The boxes were left in the dark and checked four times a day for departures of individuals, which were sexed and removed. The carcass was also checked for the presence of a feeding hole by examining the carcass for a small incision in the flesh. The timing of larval hatching was noted, and all other events were scaled relative to this event. Departure times between males and females were analysed with a *t*-test.

6.3.2 *Wild mandibles*

I caught wild beetles from the same populations in Cambridgeshire as above and bred them in a generic breeding box without exit holes (see Chapter 2). I did this in two social environments, Full Care and No Care. These two social environments were chosen because they represent the extremes of variation in parental care present in the wild, indicated by the blue (No Care) and red (Full Care) bars in Figure 6.2. In the Full Care treatment, I left parents to care and interact with their larvae throughout development. In the No Care treatment, I removed parents at ~53 hours after pairing, before larvae hatch, so that there were no interactions between parents and offspring (Boncoraglio and Kilner, 2012; Schrader, Jarrett and Kilner, 2015b). I changed the social environment to investigate the plasticity of mandible length with respect to the social environment, as many studies have noted considerable plasticity of mouthparts to, for example, host plant morphology (Carroll and Boyd, 1992; Carroll, Dingle and Klassen, 1997). Mandible length was chosen as the measure of mandible size as longer mandibles have been shown to create larger incisions during foraging in ants (Helanterä and Ratnieks, 2008). Mandible length (see Figure 6.1) and width are correlated ($N = 106$, $r = 0.63$, $P < 0.001$) suggesting that the whole mandible is likely to evolve as a unit in both length and width. Larvae were removed from the breeding box at dispersal and stored in the freezer.

Prior to dissection, each larva was weighed still frozen after removal from the freezer where they had been stored at −20°C. As wet mass and dry larval mass are highly correlated ($N = 53$, $r = 0.96$, $P < 0.001$), I used wet mass for all subsequent analyses. Larval mandibles were dissected from third-instar larvae were dissected under a dissection microscope using two entomological pins. There are no differences in mandible morphology or length between the different larval instars (Benowitz et al., 2017; Růžička, 1992). One mandible was then isolated and placed in nail polish to ensure it laid flat and its length and width

were measured using a Weiss graticule eyepiece after calibration. Measurements were done blind to the treatment and to the mass of the larva.

I used larval mass as a measure of body size and asked whether the allometric relationship between larval mandible length and body mass differs between the Full Care and No Care environments within a single generation. The best approach for estimating and comparing the allometric relationship between morphological traits is the subject of debate. Some authors have argued that since both the predictor variable (body size) and response variable (mandible length) are measured with some error, major axis regression (MA) is the best approach for estimating allometric slopes (Smith, 2009; Warton et al., 2006). However, other authors have argued that MA more generally does not provide sensible estimates of the allometric regression slopes when there are biological deviations from the allometric line, and that ordinary least squares regression (OLS) is a better approach (Egset et al., 2012; Kilmer and Rodríguez, 2016; Pélabon et al., 2014).

I employed both approaches to test for differences in the wild population in the Full Care and No Care social environments in the relationship between body size and mandible length. For all allometric analyses, all variables were ln-transformed and mandible length was cubed prior to analysis to maintain the same scale as the mass of the larva and to easy interpretation of β, where isometry is when $\beta = 1$. I used `R` 3.3.0 (R Development Core Team, 2016) for all analysis: the MA analysis was performed using the package `smatr` (Warton et al., 2012). Both OLS and MA estimates are given with the 95% confidence intervals. While I provide both estimates in tables, all figures display the results from the more conservative OLS analysis. Using MA analysis does not qualitatively change any of the results. Major axis regression was used over standardised (or reduced) major axis regression as the later involves calculating the slope as the ratio of the standard errors between both variables, a process that fails when the allometric slope is close or equal to zero.

6.3.3 *Experimental evolution populations*

The mandible allometry was estimated using two separate experimental evolution experiments. The first was performed with the populations used for the artificial selection experiment outlined in Chapter 5. The four populations that had been evolving in a Full Care environment and the four evolving in the No Care environment were treated as experimental evolution populations that had adapted to their respective social environment (Schrader, Jarrett and Kilner, 2015b), independent of the selection regime I imposed upon them. The second experiment simplified the first, in that the only selection pressures came from the

social environment, with two replicate populations in each treatment (Schrader et al., 2017). As both experiments yielded very similar results with respect to mandible allometry, I only used the second set of populations to investigate the parental evolution of carcass preparation and the possible adaptive significance of the evolution of mandible length.

The selection experiment populations

The artificial selection experiment populations had been evolving with their parents (Full Care) or without their parents (No Care) for seven generations. When I analysed mandible length the first replicates for each artificial selection treatment (see Chapter 5) were combined. All the populations were passed through a common garden generation where all larvae received parental care. This was done to minimise transgenerational effects (Kawecki et al., 2012) and to expose the genetic consequences of evolving under different social environments.

I scanned 14 larvae from the Full Care populations (Full Care 1 = 5, Full Care 2 = 9) and 26 larvae from the No Care populations (No Care 1 = 13, No Care 2 = 13), which were randomly chosen from the subset stored in the freezer. Larval mandibles were measured from 3D reconstructions of larval mandibles made using a CT scanner (XTec XT H 225 MicroCT, Nikon). Each mandible was scanned with 1000 projections with a 1 second exposure, each with a scanning power of 150kV and 97μA. The scans were complied into a 3D reconstruction using CT Pro 3D and Mimics. Measuring the length of the mandible involved taking the straight-line distance between the tip of the mandible and the outer base of the mandible. This was done to reduce error associated with the 3D reconstructed surface, as isolation of the jaws from the larva was not smooth. The tip and base of the mandibles were easily identified.

The experimental evolution populations

After the end of the artificial selection experiment (see Chapter 5), new experimental evolution populations were set up in the laboratory and exposed to either Full Care or No Care, as described in Schrader et al. (2017). Each treatment was replicated twice. The source population was created from wild-caught beetles derived from four populations in Cambridgeshire: Gamlingay Woods, Waresley Woods, Byron's Pool, and Overhall Grove. Individuals from all four populations were cross bred with offspring from all families contributing to each of the four experimental populations: two Full Care (F1 and F2) and two No Care (N1 and N2). The first replicates (F1 and N1) were on a schedule a week before the second replicates (F2 and N2).

I allowed the populations to evolve under both social environments for 24 generations, and in the 25th generation put them through a common garden

environment where all larvae received parental care, again to reduce transgenerational effects and expose the genetic consequences of 25 generations of evolution (Kawecki et al., 2012). I dissected out mandibles from the two Full Care populations (F1 = 45, F2 = 37) and the two No Care populations (N1 = 46, N2 = 40) and measured them in the same way I measured the wild mandibles.

I analysed the variation in mandible length between the wild populations and the experimental evolution Full Care and No Care populations using a Levene test in R 3.3.0 (R Development Core Team, 2016) using the package `car` (Fox and Weisberg, 2011). Mandible lengths were ln-transformed but not cubed prior to this analysis.

6.3.4 *Presence of parentally-derived holes in experimental evolution populations*

In the 13th generation of the experimental evolution populations, I randomly paired up individuals within their respective populations for both replicates, ensuring cousins and siblings did not breed. Pairs were provided with a recently defrosted mouse carcass (10–12 g). After ~53 hours, when parents are removed in the No Care populations, the carcasses they prepared were examined and the presence of a parentally derived feeding hole was noted, using the same method as for the wild pairings. I analysed the presence or absence of a hole with a binomial test.

6.3.5 *The adaptive significance of mandibles*

I performed two experiments to indirectly test the adaptive significance of mandible length. Both experiments used carcasses prepared by beetles that had been evolving with parental care for all generations to control for any potential adaptations that may have arisen in carcass preparation by the parents. Both experiments were performed after 13 generations of evolution.

Experiment 1: Carcass cutting

To test whether the presence or absence of a feeding hole in the carcass may have eroded the requirement for large mandibles, I took on the role of the parent beetles by artificially creating an incision in the carcass. Single, virgin beetles were used to prepare 120 carcasses. These individuals were derived from the stock population, which is reared in a manner similar to the Full Care populations, with the exception that wild beetles are interbred every generation in the summer months to maintain genetic diversity in the population. I used carcasses prepared by stock beetles to control for potential confounding effects of coadaptation between parent and offspring via the extended phenotype of the carcass.

Individuals were given a recently defrosted mouse carcass (8–14 g) and left to prepare the mouse for 68 hrs. This is longer than the time allotted before removal of parents in the experimental populations, but for a single individual the extra time compensates for a lack of partner (Trumbo, 1992). Each carcass was randomly allocated to one of two treatments: Cut or No Cut. The Cut treatment consisted of a 8 mm incision into the thigh of the hind leg of the mouse and was designed to be as similar as possible to the cut inserted by the parental beetles (Eggert, Reinking and Müller, 1998), all done without unravelling the spherical carcass. All carcasses were examined before any treatment occurred for the presence of a parentally-made hole. As expected for virgins, few holes were present, and carcasses with any incisions were not used for the experiment.

Pairs of Full Care ($F_1 = 15$, $F_2 = 35$) and No Care ($N_1 = 20$, $N_2 = 35$) beetles were provided with a recently defrosted 24–26 g mouse to induce larger clutches from which we could extract first-instar larvae. Parents were left for ~53 hours, until the clutch was laid. At this time, the carcass was removed and replaced with a small quantity of beef mince to ensure larvae did not die. The boxes were checked every eight hours for newly hatched larvae. I randomly collected first-instar larvae from the breeding boxes into one petri dish. From this mix, I placed ten larvae directly on to a randomly chosen carcass.

The experiment therefore had four treatments: Full Care Cut ($N = 24$); Full Care No Cut ($N = 23$); No Care Cut ($N = 33$); and, No Care No Cut ($N = 33$). The newly hatched larvae from each population were pooled and randomly allocated to a Cut or No Cut carcass in broods of 10 where they were left without parents. Broods were weighed at dispersal, which was defined as when two or more larvae were observed crawling away from the carcass (Rauter and Moore, 2002). Brood size and brood mass data were collected at dispersal.

To analyse Experiment One, I used generalised linear mixed-effects models using the `lme4` package (Bates et al., 2015) in `R` 3.3.0 (R Development Core Team, 2016). The number of surviving larvae was analysed using a generalised linear mixed effects model with binomial error structure, as data were bounded by 0 and 10. Carcass mass and sex of the preparing beetle were included as covariates, with block as a random term. The number of successful broods (with at least one surviving larvae) was analysed using Fisher's exact test.

Experiment 2: Smallest surviving larvae

Experiment Two followed the same protocol as Experiment One, but with two differences. Ten larvae were placed on stock-prepared carcasses without an artificial incision, prepared by a male and female. In all other aspects Experiments One and Two were identical and performed during the same time period. The larvae were placed on the carcass without parental care and were weighed in-

dividually at dispersal. This way, I could identify the variance of the surviving larvae and identify the smallest surviving larvae from each brood. The smallest surviving larva from each brood from the Full Care population was compared with the smallest surviving larva from each brood from the No Care population using a *t*-test in R.

6.4 RESULTS

6.4.1 *Variation in parental care*

In general, the variation in departure time of parental beetles was large (Figure 6.2). Males tended to leave earlier than females ($t_{66} = 4.07, P < 0.001$), and in two out of 34 cases (6%) both parents left the breeding chamber before larvae had hatched. Larvae from one of these pairs completed development. This indicates that some parents may abandon their offspring in the wild. This estimate is conservative, as the beetles were reared in a lab environment without environmental perturbation, or risk of usurpation, which likely further reduces the length of parental care (Robertson, 1993; Trumbo, 1990). There was also evidence that the timing of the incision into the carcass may vary. In nine out of 34 cases (26%) the incision was made prior to the arrival of the larvae (Figure 6.2).

6.4.2 *Mandible allometry in wild beetles*

There was no evidence of plasticity on the mandible allometry for the wild larvae, as measured by the interaction between body size and social environment (OLS: $t_{103} = -1.21, P = 0.23$; MA: $LR_1 = 1.55, P = 0.21$, Figure 6.3). The allometric slopes for larvae reared in both Full Care and No Care environments were not different from zero (Table 6.1), for both OLS (Full Care: $t_{52} = 1.60, P = 0.12$; No Care: $t_{52} = -0.11, P = 0.91$; Combined: $t_{106} = 1.58, P = 0.12$) and MA regression (Full Care: $r_{52} = 0.22, P = 0.12$; No Care: $r_{52} = -0.02, P = 0.91$; Combined: $r_{106} = 0.15, P = 0.12$).

6.4.3 *Mandible allometry of the experimental evolution populations*

I found evidence that the mandible allometry had evolved in both the selection and experimental evolution populations (Figures 6.4 and 6.5). There was a significant interaction between the treatment and body size with respect to mandible length in the selection experiment (Figure 6.4, OLS: $t_{23} = -2.65, P = 0.02$; MA: $LR_1 = 6.18, P = 0.01$). The allometric slopes also differed from a slope of zero. The Full Care-evolved populations had a steep slope (Table 6.2) that was different

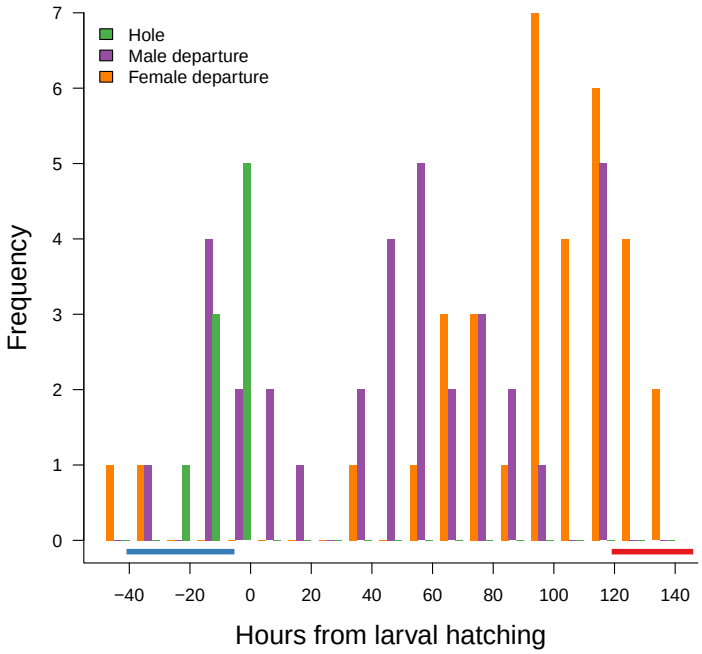


Figure 6.2 | The natural variation in the time spent looking after larvae, and the incision of a feeding hole in the carcass. Data were collected in the lab from wild beetles. All the data are scaled relative to the timing of larval hatching, at hour 0 on the x axis. Male and female departure is when the male or female left the breeding part of the box. The hole indicates at what point a feeding hole is visible in the carcass. The timing of the hole often corresponded to when the larvae arrived; the plot, therefore, shows only the data when the hole was observed prior to larval hatching. In two cases ($N = 34$) both parents left prior to larval hatching. Larvae completed development in only one of these cases. The bars at the bottom indicate the timings of our experimental manipulations relative to the variation in hatching time. The blue bar indicates the No Care treatment, with the red bar indicating the Full Care treatment.

from zero (OLS: $t_{11} = 3.93, P = 0.002$; MA: $r_{11} = 0.75, P = 0.002$). The No Care-evolved population also had an allometric slope that differed from zero (Table 6.2, OLS: $t_{23} = 2.63, P = 0.014$; MA: $r_{23} = 0.47, P = 0.014$).

I replicated these data with the experimental evolution populations (Figure 6.5). Again, I found a significant interaction between the evolutionary history of the populations and body size in the length of the larval mandibles (OLS: $t_{163} = -2.87, P = 0.005$; MA: $LR_1 = 9.65, P = 0.002$). The slopes also differed from a slope of zero (Table 6.2), both in the Full Care populations (OLS: $t_{80} = 11.39, P < 0.001$; MA: $r_{80} = 0.79, P < 0.001$), and the No Care populations (OLS: $t_{83} = 11.53, P < 0.001$; MA: $r_{83} = 0.78, P < 0.001$). The allometries of both the experimental evolution Full Care ($t_{177} = -5.68, P < 0.001$) and No

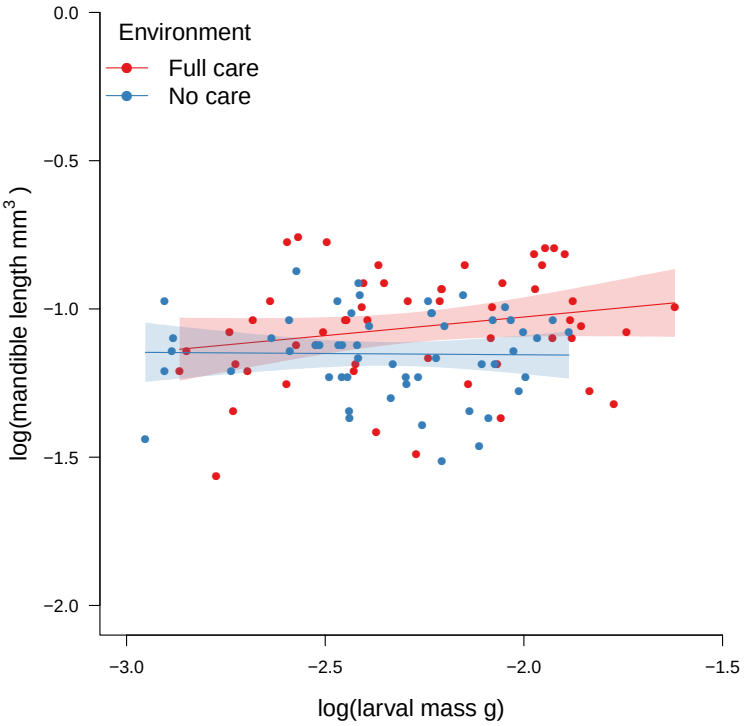


Figure 6.3 | The allometric relationship of mandible length to body mass in wild-derived larvae. The allometric slopes did not show plasticity for the social environment. There was no difference in the mandible allometry if the larvae were reared with post-hatching parental care (red) or without post-hatching parental care (blue). Lines shown are OLS slopes with 95% confidence intervals. The slopes for both social environments and when combied were not significantly different from zero.

Table 6.1 | The allometric slopes (β) of the relationship between larval mass and the cube of mandible length of wild larvae in two social environments: Full Care and No Care. Both ordinary least squares (OLS) and major axis (MA) regression estimates are listed with the 95% confidence intervals in parentheses below.

Environment	OLS	MA
Full Care	0.126	0.183
	(−0.032, 0.283)	(−0.049, 0.435)
No Care	−0.009	−0.012
	(−0.158, 0.141)	(−0.234, 0.208)
Combined	0.088	0.130
	(−0.022, 0.198)	(−0.034, 0.301)

Care ($t_{182} = -2.68, P = 0.008$) populations significantly differed from the Wild mandible allometry.

Table 6.2 | The allometric slopes of the relationship between larval mass and the cube of mandible length of larvae that have evolved under two social environments: Full Care and No Care. Mandibles were measured under the same common garden social environment of Full Care. Data from the selection experiment and experimental evolution populations are shown. The selection experiment data was collected using CT-scans whilst the experimental evolution data was measured from dissected mandibles. Both ordinary least squares (OLS) and major axis (MA) regression estimates are listed with 95% confidence intervals below.

Population	Selection		Experimental evolution	
	OLS	MA	OLS	MA
Full Care	0.901	1.275	0.382	0.414
	(0.529, 1.272)	(0.716, 2.501)	(0.315, 0.448)	(0.343, 0.488)
No Care	0.300	0.421	0.266	0.278
	(−0.160, 0.760)	(0.102, 0.836)	(0.220, 0.312)	(0.230, 0.326)

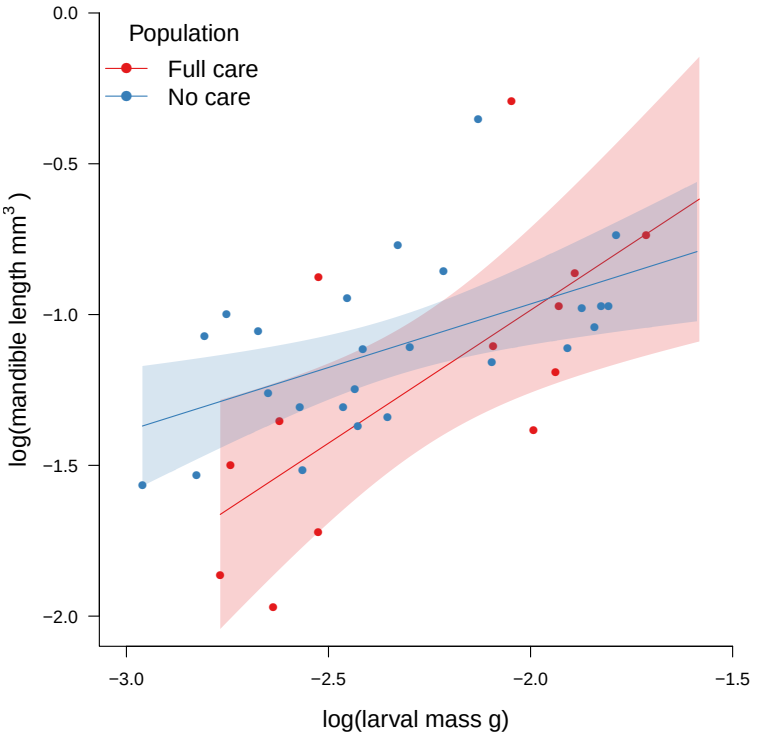


Figure 6.4 | The mandible allometry after seven generations of evolution during the selection experiment outlined in Chapter 5. The No Care populations (blue) have a more negative allometry than the Full Care populations (red). Slopes shown are OLS estimates with 95% confidence intervals.

The overall variance of absolute mandible length, without controlling for body size, differed significantly between the Full Care and the No Care popula-

tions ($F_{1,165} = 12.86, P < 0.001$), whilst the body mass distribution between the populations did not differ ($F_{1,166} = 0.002, P = 0.98$).

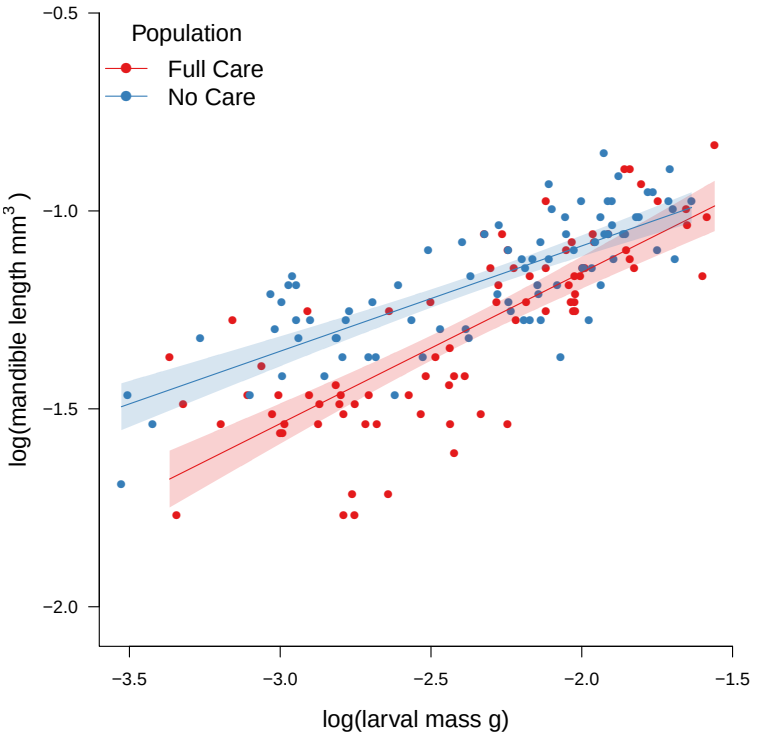


Figure 6.5 | Mandible allometry for the experimental evolution populations where mandibles were dissected out and measured using a compound microscope. The allometric slopes are different between the social environments populations have been evolving under. OLS slopes with 95% confidence intervals are shown.

6.4.4 *Parentally-derived incisions*

Parents from the No Care populations were more likely to make an incision into the carcass prior to larval hatching, than individuals that had evolved with parental care ($z = 5.28, P < 0.001$, Figure 6.6). The proportion of Full Care-prepared carcasses with an incision (F1 = 27%, F2 = 32%) was very similar to the proportion of wild beetle-prepared carcasses that had an incision before larval hatching (26%, Figure 6.2). The No Care populations had a much larger proportion of carcasses with parentally-derived incision (N1 = 60%, N2 = 62%).

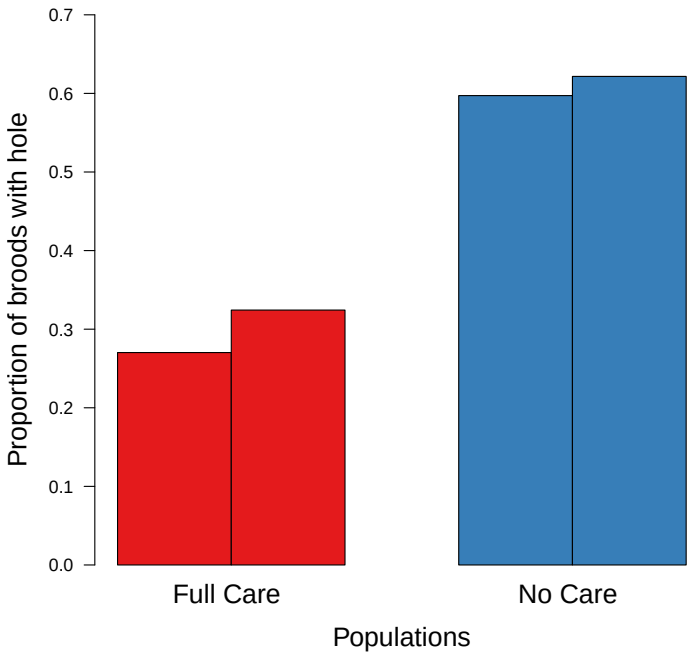


Figure 6.6 | The proportion of breeding pairs from all four experimental evolution populations (two Full Care in red, and the two No Care in blue) that made an incision into the carcass prior to larval hatching, ~ 53 hours after being paired up.

6.4.5 *The adaptive significance of mandibles: experiment 1*

There was a significant interaction of brood success between the Cut treatment and the evolutionary history of the larvae ($P < 0.001$). No broods failed in the Cut treatment, independent of the social environment in which they evolved. However, more broods were successful if they were made up of No Care larvae in the No Cut treatment. There was no interaction of the population and the treatment on the number of surviving larvae ($z = 1.096, P = 0.273$). There were, however, independent, additive effects of both (Figure 6.7a). When on a Cut or a No Cut carcass, more No Care larvae survived ($z = 8.032, P < 0.001$). In addition, more larvae from both social environments survived on a Cut carcass ($z = 9.074, P < 0.001$).

6.4.6 *The adaptive significance of mandibles: experiment 2*

The smallest surviving larva on an uncut carcass was smaller for No Care broods than Full Care broods (Figure 6.7b, $t_{37} = -2.22, P = 0.03$).

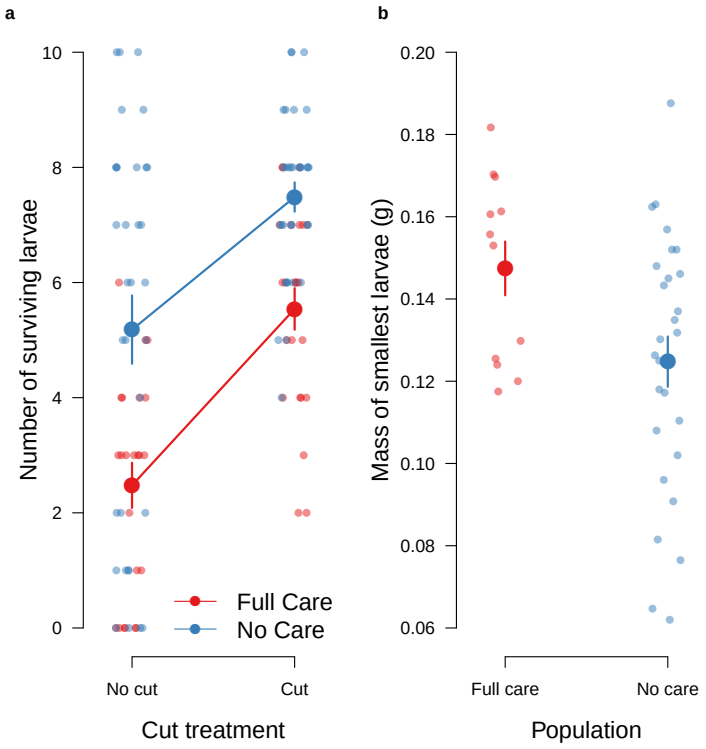


Figure 6.7 | Data from two experiments designed to test the potential adaptive significance of the evolved mandible allometry. **a** More No Care larvae than Full Care larvae survived on a No Cut carcass and a Cut carcass. On a Cut carcass, the number of Full Care larvae that survived increased to the sam number of No Care larvae that survived on a No Cut carcass. Interestingly, the number No Care larvae that survived also increased on a Cut carcass. **b** The smallest larva that survived from each brood on a carcass without an artificial incision was smaller for No Care larvae, which would be expected if a threshold mandible length is required for larval fitness. In both panels, means are shown with standard errors.

6.5 DISCUSSION

Here, I have identified an offspring adaptation in wild burying beetles that has evolved under fluctuating selection in a social environment. I hypothesised that larval mandibles are key to larval fitness, as they aid the larvae in accessing the resources within the carcass, as well as self-feeding once inside. When parents care for their offspring post-hatching, they help in both these cases by chewing a hole into the carcass and regurgitating pre-digested carrion to the larvae (Smiseth and Moore, 2002b). My first experiment suggests that in the wild, levels of post-hatching care are unpredictable; larvae do not know how much help they will receive in accessing the carcass or in feeding (Figure 6.2). To counter this unpredictability, larvae appear to bet hedge; that is, they adopt a single strategy

that does best in either environmental extreme, namely in a size-independent mandible length—a highly unusual negative allometry (Figure 6.3).

Strikingly, the mandible allometry becomes less negative in larvae which had evolved under predictable levels of parental care ($\beta > 0$), indicating that mandibles are an adaptation to the predictability of the social environment. The positive allometry in the Full Care populations may be a direct result of the predictable level of care received by the larvae, so that small larvae do not need to compensate for the absence of parental care by developing disproportionately large mandibles for accessing the carcass and for feeding. The No Care population allometry also evolved to be more positive, albeit to a lesser extent, at odds with this interpretation (Figures 6.4 and 6.5). Nevertheless, two arguments speak in favour for a key difference between the two treatments. First, concurrent evolution of parental behaviour can account for this erosion from $\beta = 0$ in the No Care treatment, as No Care parents simultaneously evolved to make the environment less harsh for their offspring by chewing a hole into the carcass prior to larval hatching (Figure 6.6). Second, using experimental manipulations of the carcass, I showed that the difference between the Full Care and No Care allometries is large enough to have a significant impact on larval fitness (Figure 6.7).

The social environment created by parents has important consequences for the fitness of their offspring. Variation in parental care exhibited by *N. vespilloides* likely exerts fluctuating selection on their offspring (Figure 6.2). When selection acts in different directions across generations, phenotypic plasticity has been hypothesised to evolve in some parameter space (Hallsson and Björklund, 2012; Tufto, 2015). Insect mouthpart morphology is particularly prone to display phenotypic plasticity, presumably due to the unpredictable nature of food resources (Carroll, Dingle and Klassen, 1997; Carroll, Klassen and Dingle, 1998; Pappers, Velde and Ouborg, 2002). Recent work has shown that mandible morphology in a beetle, *Gnatocerus cornutus*, is under epigenetic control, and hence has the capacity for plasticity (Ozawa et al., 2016). I did not find, however, any evidence of plasticity in mandible size of burying beetle larvae in relation to the social environment in which they found themselves (Figure 6.3). Tufto (2015) stated that under fluctuating selection, when autocorrelation is low and residual differences from the optimal phenotype in all environments are small, canalising selection could occur, a conclusion backed up by theory on genetic canalisation (Kawecki, 2000). I provide evidence for this hypothesis with the wild data and by evolving populations under predictable care regimes: mandible length was canalised with respect to its environment, body size, as a result of the unpredictable social environment. The occurrence of a size-independent mandible length in wild larvae could be a bet-hedging strategy; a "one-size-fits-all" scenario where a single

particular mandible length does best by reducing variation in the geometric mean fitness of offspring across all possible environments (Philippi and Seger, 1989; Simons, 2011; Slatkin, 1974).

By evolving populations under predictable social environments, I have shown that the flat mandible allometry disappears and can therefore be attributed to more unpredictable levels of parental care. The mandible allometry of both Full Care and No Care populations evolve to be more positive ($\beta > 0$), with the Full Care allometry significantly more positive than the No Care allometry (Figures 6.4 and 6.5). In addition, this result was obtained in two independent experiments, though to different extents, which may be due to the difference in sample size and the number of generations each population was evolving. The selection experiment imposed selection on adult body size that may have confounded the evolution of larval mandibles, but provided evidence of mandible evolution after only seven generations. The speed of allometric evolution suggests that allometric scaling relationships need not be a constraint on subsequent morphological evolution (Bolstad et al., 2015; Voje et al., 2013), but instead could be the origin of morphological novelty (Nijhout and McKenna, 2017). Flat allometries, through canalising selection, may harbour large amounts of genetic variation that could contribute to rapid evolution. Previous studies have suggested that allometric intercepts are thought to have a greater evolvability, as they respond more rapidly to artificial selection than allometric slopes (Egset et al., 2012; Voje et al., 2013; but see Tobler and Nijhout, 2010), and show greater variation than slopes between species (Pélabon et al., 2014). I found, however, that larval mandible evolution involved a shift in the allometric slope (β) and not just a shift in the allometric intercept ($\log(\alpha)$), which could indicate allometric slopes are easier to evolve that previously thought. Whilst the mandible allometry has evolved between the two experimental populations, whether they have both originated from the flat allometry of the wild would have required measuring the allometry at generation one. The flat allometry was measured from wild individuals from the populations that started the experimental lines, but only one generation after the initiation of the experimental evolution lines. Further planned work to interrogate this further is using fluctuating selection lines where previously evolved populations will be exposed to random fluctuations of care and no care, with the prediction being the mandible allometry will evolve towards a slope of zero.

Elucidating the genetic and developmental mechanisms behind allometric scaling relationships can therefore give insights into the potential evolvability of morphological traits (Emlen et al., 2012; Nijhout, 2003; Nijhout and Grunert, 2010). Shifts in the allometric intercept (e. g. Frankino et al., 2005) arise through changes in the development time of traits and are under the control of the hormone ecdysone (Nijhout, 2003; Nijhout, Davidowitz and Roff, 2006; Stern

and Emlen, 1999). The allometric slope, however, is heavily linked with the growth rate of imaginal disks within holometabolous insects, which in turn is developmentally tied to the insulin pathway, the degree to which organs are sensitive to insulin (Emlen, Lavine and Ewen-Campen, 2007; Emlen et al., 2012; Shingleton et al., 2007), and potentially the interaction of the insulin pathway with ecdysone (Colombani et al., 2005). Despite the hypothesis that allometric slopes are more constrained in their evolution, social traits, however, show considerable range in positive allometric slopes across species (Kodric-Brown, Sibly and Brown, 2006). The vast array of positive allometric slope estimates indicate that at a macroevolutionary level β can evolve free from constraint (Labonte et al., 2016). Artificial selection on trait slopes, however, fail to yield a response (Egset et al., 2012; but see Tobler and Nijhout, 2010). The predisposition for social traits to evolve β indicates that social selection may be required for such evolutionary change. In this chapter, I show that a simple change of the social environment readily induces evolution of the allometric slope. Whether morphological traits which are under the direct influence of the social environment have greater evolvability of their slopes remains to be seen.

I found that consistent, stabilising selection imposed by a predictable social environment led to the evolution of increased sensitivity of larval mandibles to body size. This potentially involved changes in the nutritional environment of larval size due to sensitivity in the insulin pathways (Emlen, Lavine and Ewen-Campen, 2007; Emlen et al., 2012; Shingleton et al., 2007), and would be a clear genetic candidate for differences between the experimental evolution populations. But in addition to this likely increased sensitivity to nutrition, the total phenotypic variation of mandible lengths differed between the Full Care and No Care populations. Independent of body size, phenotypic variation was smaller in the No Care populations. In *Drosophila*, variation in traits linked to higher fitness (e. g. genitalia) is lower (Stearns and Kawecki, 1994). This suggests that first, canalising selection has acted on mandible length in the No Care and wild lines, and second, that it is a trait of greater importance to the No Care larvae than the Full Care larvae. Notably, there is a large amount of phenotypic variation in mandible length in the small larvae (Figure 6.5), suggesting that the Full Care environment has weakened selection on mandible length, but also that it maintains this variation. By chance, parental care could therefore facilitate rapid evolution should environments change (see Chapter 4).

The No Care mandible allometry also evolved in concert with evolutionary change in the social environment itself. I predicted the No Care allometry would remain flat, as parents were never present to aid larval feeding, but the allometry did evolve and erode from $\beta = 0$ (Figure 6.5). The parental beetles also evolved in this social environment. The No Care parents were more likely to make a

feeding incision into the carcass prior to larval hatching (Figure 6.6), in effect reducing selection for disproportionately large mandibles when larvae are small, but not completely removing it. While this was an unexpected result, it is not a surprise. The social environment contains genes which theoretically should increase the rate of evolution of both parents and offspring through feedback loops (McGlothlin et al., 2010; Moore, Brodie and Wolf, 1997; Wolf, Brodie and Moore, 1999), similar to those in Chapter 5 and Jarrett et al. (2017). Indirect genetic effects create coevolutionary feedbacks within the family, where the offspring are products of the parents, and the parents are shaped by their offspring (Darwin, 1859). Coadaptation between parents and their offspring is also a likely scenario when the social environment contains genes, where genetic combinations between parents and offspring are matched to maximise fitness of both parties (Agrawal, Brodie and Brown, 2001; Hinde, Johnstone and Kilner, 2010; Wolf and Brodie, 1998). It would be interesting to test whether this is the case for mandible length and parental departure time; perhaps offspring with large mandibles have parents that care less and depart the carcass earlier.

I determined the function of the evolved mandible allometry between the Full Care and No Care populations using two experiments, the results of which aligned with my predictions. By cutting a hole in to the mouse, simulating the action of the parents (Figure 6.6), more Full Care larvae survived; in fact, the same number as from the No Care populations when no cut was present (Figure 6.7a). Access into the carcass is therefore a strong selection pressure that appears to be selecting for longer mandibles. When the carcass was cut, still more No Care larvae survived. This is an indication of potentially a further adaptation in the No Care offspring. Either the larger mandibles, as predicted, confer a greater advantage in self-feeding, or the interactions between the No Care larvae are more cooperative. The other prediction based on the mandible allometry is that smaller No Care larvae have disproportionately larger mandibles than a similarly sized Full Care larvae, leading to a disparity in the size of the smallest surviving larva on a carcass if indeed mandible length is important for survival. I found exactly this, where the smallest surviving larva in No Care broods was smaller than their Full Care equivalents (Figure 6.7b).

Most traits exhibit prolonged periods of evolutionary stasis (Eldredge et al., 2005; Hansen and Houle, 2004), and yet here I show rapid evolution of an allometric relationship through an evolutionary gain in plasticity. Perhaps micro-evolutionary change commonly reported in laboratory and field studies reflects fluctuations of which traits gain (e. g. this chapter, Suzuki and Nijhout, 2006) or lose (e. g. Levis and Pfennig, 2016) sensitivity to wider environmental conditions. Whether these gains or losses of plasticity also contribute to larger and more

infrequent patterns of macroevolutionary change will prove much more difficult to determine.

Part IV

PARENTS IN THE WIDER WORLD

7

PROVISIONING BEHAVIOUR BY PARENTS: FROM MICROEVOLUTION TO MACROEVOLUTION

7.1 SUMMARY

In this chapter, I summarise the results I have presented in this thesis on the role of parental care in the evolution of burying beetles. I then broaden my focus to analyse the role of parental provisioning in macroevolutionary patterns of speciation. I use sister-clade comparisons to show that clades that exhibit provisioning behaviour are likely to have more species than clades that do not exhibit provisioning behaviour. There are many potential, non-mutually exclusive mechanisms underpinning this pattern, which I discuss.

7.2 INTRODUCTION

Parental investment is widespread in sexually reproducing organisms, often in the form of care. Parents invest in time and resources to produce offspring, which includes the care of eggs, protection of young, or feeding their young prior to, or after, hatching (Clutton-Brock, 1991). How and why parental care has evolved has been the subject of extensive study (see Chapter 1; Clutton-Brock, 1991; Gilbert and Manica, 2015; Royle, Smiseth and Kölliker, 2012; Wong, Meunier and Kölliker, 2013). But the question of what happens after parental care evolves has received little attention (Badyaev and Uller, 2009).

Throughout this thesis, I have used a variety of methods to investigate the role of parental care in the evolutionary process. I have focused on one genus, *Nicrophorus*, to better understand this link, and used only one species, *N. vespilloides* for my experimental work. The *Nicrophorus* genus is much more species rich than its sister genus, *Ptomascopus*, which does not exhibit the same elaborate care that *Nicrophorus* parents show. Throughout my thesis, I have attempted to gain an understanding of whether the evolution of parental care in this genus facilitated the radiation of the burying beetles, and what mechanism may have driven it.

In Chapter 3, I used a combination of experiments with two *N. vespilloides* populations and comparative analyses to understand what ecological and community-level forces shaped the radiation of the *Nicrophorus* genus. Guilds of burying beetles contain several species that appear to be sorted by body size, where each

guild has a large species, several medium species, and one or two small species (Scott, 1998b). Does body size reduce competition between species, enabling co-existence between sympatric species? And by what mechanism does this occur? To answer these questions, I made use of two populations in Cambridgeshire that differ in their species compositions (S.-J. Sun, unpublished data). I looked at the effect of the shift in competition caused by the contrasting guild structures in the two populations. I found, contrary to my predictions, that body size did not differ between the populations, where greater competition from the two medium sized species may select for smaller-sized *N. vespilloides*. Instead, the two *N. vespilloides* populations differed in how they utilised the carrion niche (Figure 3.4). The population that experienced the greatest level of competition produced larger larvae on carcass sizes that they likely compete for with the larger species. Larger parents produce larger offspring on a medium carcass if they have been evolving with greater competition (Figure 3.6). How a niche is partitioned to reduce interspecific competition is sometimes not obvious. Analysis of body size between the populations would not indicate whether the increased competition has influenced the evolution of traits to reduce competition, or how it could have done so. Behaviour is likely the trait that responds first to the environment, including the level of interspecific competition, and may result in subsequent morphological change. Analysis of just morphology in this case, would have led to the conclusion that interspecific competition does not influence the evolution of these burying beetle populations.

In combination with laboratory experiments, I found evidence that body size evolution is driven by interspecific competition using a global comparative dataset across the whole *Nicrophorus* genus. More closely related species in sympatry were more diverged in their body sizes than the closest related species in allopatry (Figure 3.7). Further to this, I found that larger species were more dependent on parental care for offspring survival (Figure 3.8). In Chapter 3, I find that body size is the trait by which the *Nicrophorus* genus has diversified, through partitioning of the carrion niche, and that the evolution of parental care is likely the agent that facilitated the adaptive radiation.

In Chapter 4, I build on the results from Chapter 3 by investigating the evolutionary potential of populations under different social and resource environments. As both the social environment provided by parents and the size of the carrion appear to be important in the evolution of *Nicrophorus* in the wild, I combined both in a quantitative genetic framework. In so doing, I tested how the additive genetic variation (V_A) changes across environments, which addressed whether the removal of parental care reveals previously cryptic genetic variation which would manifest as greater V_A. I instead find that there is no shift in V_A between any environments in the three traits I measured (Tables 4.2, 4.3 and 4.4).

Intriguingly, as larval mass, development time, or pronotum width do not appear to have any additive genetic variation, classical quantitative genetic models of evolution predict they should not evolve. The results from this chapter indicate the most likely method for evolution in these traits is through IGEs, as maternal and sibling effects were considerable.

Chapter 5 tests the idea that parents can facilitate evolutionary change in body size even without significant heritability of adult pronotum width. I used an artificial selection experiment to show that understanding multiple social factors is important in determining the evolvability of a trait. I found that populations only responded to selection for large body size when parents were there to care for their offspring (Figure 5.2). Conversely, populations only responded to selection for small body size when parents were absent. Populations did not respond to selection for large body size without parents, and small body size when parents cared for their offspring (Figure 5.2). Three social factors interacted to determine the response to selection: clutch size, brood size, and the larval size–brood size trade-off (Figure 5.6). Large size can only be achieved when parents cooperate with offspring by provisioning small broods. Small body size can only be achieved across evolutionary time because of the cooperative nature of sibling interactions when parent are absent (Schrader, Jarrett and Kilner, 2015a). Negative interactions, like sibling conflict, appear to retard the response to selection in artificial breeding designs (Bergsma et al., 2008; Bijma and Wade, 2008; Camerlink et al., 2015; Wade et al., 2010). My work, together with other microevolutionary studies, shows that conflict slows the pace of evolution, whereas cooperation accelerates it.

Parents not only shape how populations respond to selection, but can also impose selection themselves. The social environment parents create is an environment that selects for offspring traits, which feed back into the evolution of their parents and the social environment they create. As Darwin (1859) states: "*Natural selection will modify the structure of the young in relation to the parent, and of the parent in relation to the young*". The populations I used for the selection experiment evolved in two different social environment, which showed rapid and replicable adaptation to those social environments (Schrader, Jarrett and Kilner, 2015b; Schrader et al., 2017). In Chapter 6, I followed separate experimental evolution populations and identified a likely larval adaptation that has evolved in response to a change in the social environment induced by parents. Larval mandibles are crucial for larval survival on a carcass without their parents, which in the wild could be any breeding attempt. In response to an unpredictable social environment (Figure 6.2), the larval mandible allometry is flat (Figure 6.3). Rapid morphological evolution follows where small larvae have evolved smaller mandibles in both the No Care and Full Care populations, though, as predicted,

the No Care larvae have larger mandibles than the Full Care (Figure 6.4). Again, I show the advantage of experimental evolution approaches to understanding dynamics between parents and their offspring. The No Care mandible allometry also shifted, which I did not predict. What I did not predict was the coevolutionary process that occurs between parents and offspring, whereby the parents have also evolved to make a feeding incision prior to larval hatching, thus reducing selection acting on their offspring to access the resources within the carcass. Just as Darwin predicted, both the parents and the offspring modify each other.

7.3 MODES OF PROVISIONING

Here, I expand the idea that parental care is a driver of evolutionary change by asking whether there is a large scale pattern of macroevolutionary diversification that is correlated with the evolution of more elaborate parental care. To do so, I found examples of post-hatching provisioning behaviour across invertebrate taxa, and asked whether a greater number of species is found in clades that do exhibit such behaviour when compared with sister taxa that lack offspring provisioning. I start by outlining why I have focused on parental provisioning behaviour, and in doing so, compare it with viviparity. The evolution of viviparity has been implicated in further diversification and mirrors the hypothesis that behavioural provisioning accelerates evolutionary change. The mechanisms behind both may be similar. Next, I compare the number of species found in sister clades, when one exhibits parental provisioning behaviour whilst the other does not. I find that clades that have evolved provisioning behaviour have more species than clades that have not evolved provisioning. Lastly, I discuss the potential mechanisms that may be driving this pattern.

7.3.1 *Oviparity and viviparity*

Resource provisioning by parents to their offspring has evolved many times across many different taxa (Clutton-Brock, 1991). The act of parents providing nutrients for offspring development is perhaps not as complex a trait as we might imagine: egg-laying organisms, and trees and plants, provision their eggs or seeds with a nutritious yolk or endosperm that provides the resources required for complete offspring development. Oviparity has often evolved into viviparity—the birthing of live young—in insects, fish, reptiles, and mammals (Clutton-Brock, 1991; Pyron and Burbrink, 2014). Viviparous species across all taxa display a diverse array of provisioning offspring whilst they are retained within the mother. Developing young can feed on their siblings by eating undeveloped eggs or other embryos; from the mother's own soma; through specialised secretions derived

from the mother; or, most commonly, directly form the mother's blood, through a placenta (Clutton-Brock, 1991).

Animal families and the evolution of parental care may appear cooperative, as both parents and offspring jointly benefit from greater investment into offspring. Insights from kin selection (Hamilton, 1964), demonstrate that these interactions may not be as cooperative as they appear. Trivers (1974) observed that parents in monogamous pairs are equally related to all their offspring and so should invest equally in all of them ($r = 0.5$). Their offspring, however, are more related to themselves ($r = 1$) than they are to their full siblings ($r = 0.5$). Offspring are therefore selected to demand more resources from their parents than their parents are selected to provide. Conflict between parents and their offspring occurs only when parents and offspring interact, which is not possible during the development of the egg in oviparous species.

Zeh and Zeh (2000, 2008) hypothesised that the conflict arising between mother and offspring in viviparous species, where offspring can influence the supply of resources from the mother, can facilitate speciation. The viviparity-driven conflict (VDC) hypothesis posits that the conflicts arising between maternal and offspring genomes result in continuous antagonistic coevolution. Different resolutions to these conflicts could arise but they will be incompatible with one another, increasing the rate of post-zygotic isolation and ultimately speciation (Zeh and Zeh, 2000, 2008). Experimental evidence supports the predictions of the VDC hypothesis. For example, a greater placental invasiveness results in greater hybrid failure in mammals (Elliot and Crespi, 2006). In fish, Schrader and Travis (2008) showed greater rates of abortion in crosses between monandrous and polyandrous populations of *Heterandria formosa*, a matrotrophic poeciliid. The different mating systems between the populations influences the likely relatedness between siblings. This changes selection on offspring to demand more from their mother (the mother is equally related to all her offspring independent of the sire). The asymmetry of abortions was in the direction predicted by the VDC hypothesis—monandrous females aborted more when mated with polyandrous males (Schrader and Travis, 2008). The VDC hypothesis is also consistent in results gathered through comparative methods. For example, clades of ray-finned fishes that had evolved viviparity also had greater species richness (Mank and Avise, 2006).

7.3.2 *Mass and progressive provisioning*

There are parallels between parity mode and the behavioural methods of provisioning offspring. Oviparous species invest heavily in egg contents before offspring hatch, while mass provisioning organisms have a stocked larder for

their unhatched eggs. Mass provisioning likely evolves when resources are spatially distributed or ephemeral (Gardner and Smiseth, 2011), like balls of dung provided by dung beetle parents (Hanski and Cambefort, 1991), or caterpillars and spiders like *Ammophila* wasps (Field, 1992). By extension, progressive provisioning is more analogous to viviparity because parents continually provide resources throughout offspring development. In viviparous species this is mediated through a placenta, but in progressive provisioning species parents and offspring interact behaviourally to determine the resources provided by the mother to the offspring. Progressive provisioning likely evolved in response to the benefits of shepherding offspring through development (Clutton-Brock, 1991; Field, 2005), when they are threatened by harsh environments, predators, or parasites (Field and Brace, 2004). Adopting a strategy like progressive provisioning provides the opportunity for further parental effects beyond that of the egg or ball of dung. Just as the VDC hypothesis predicts greater subsequent rates of diversification, progressive provisioning might also drive patterns of macroevolution.

7.4 PROGRESSIVE PROVISIONING IN SPECIATION

Throughout this thesis, I have shown that parental care in a progressively provisioning species can lead to rapid microevolutionary change in populations. But does progressive provisioning result in macroevolutionary patterns of greater divergence and ultimately greater diversification? To test the prediction that the evolution of progressive provisioning leads to an increase in species diversity, I used sister clade comparisons across all arthropods. This involves comparing the two most closely related clades, where one clade shows evidence for progressive provisioning and the other clade does not. Comparing the number of species within each clade then means I can test whether there is an association between the extent of parental care and the rate of evolution.

7.4.1 *Classifying clades with provisioning*

I used the following methods to identify clades that exhibited progressive provisioning behaviour. I first used books and literature reviews (Choe and Crespi, 1997; Clutton-Brock, 1991; Costa, 2006; Hunt and Nalepa, 1994) to list known cases of species that progressively provision their offspring. Secondly, I searched the literature that had cited all of the above books on Google Scholar to further identify cases known after the publication of the book. Thirdly, I used search strings and terms in a consistent manner across Google Scholar and the Web of Knowledge databases. Search strings consisted of "parental care", "progressive

Table 7.1 | Data concerning identified taxa that exhibit provisioning behaviour as parents and their respective sister taxa. Orders are shown in bold.

Provisioning		Non-provisioning				
Taxon	Spp.	Taxon	Spp.	Provisioning Reference	Species Reference	Phylogeny
HIRUDINEA						
Heloddella	56	*Haementeria*	10	Kutschera and Wirtz, 2001	Christoffersen, 2009	Christoffersen, 2009
ARANEAE						
Erisidae	96	Oecobiidae + Hersiliidae	291	Yip and Rayor, 2014	Platnick, 2014	Coddington, 2005
Theridiidae	2387	Nesticidae	221	Yip and Rayor, 2014	Platnick, 2014	Coddington, 2005
Agelenidae	1223	Desidae + Amphinectidae	334	Yip and Rayor, 2014	Platnick, 2014	Coddington, 2005
COLEOPTERA						
Nicrophorus	63	*Ptomascopus*	4	Scott, 1998b	Sikes, Madge and Newton, 2002	Sikes and Venables, 2013
Bledius	439	*Eppelsheimius*	2	Costa, 2006; Larsen, 1952	Herman, 1986	Herman, 1983
Scolytinae	6000	Cyladinae	24	Clutton-Brock, 1991	Capinera, 2008; Sforzi and Bartolozzi, 2004	Hunt et al., 2007
Passalidae	500	Trogidae	300	Clutton-Brock, 1991	Foottit and Adler, 2009	Hunt et al., 2007
Platypodinae	1500	Cossoninae	1700	Costa, 2006	Capinera, 2008	Hunt et al., 2007

Table 7.1 – continued from previous page

Provisioning		Non-provisioning				
Taxon	Spp.	Taxon	Spp.	Provisioning Reference	Species Reference	Phylogeny
Dermaptera						
Forficulidae	461	Chilisochidae	95	Costa, 2006; Wong, Meunier and Kölliker, 2013	Deem, 2014	Jarvis, Haas and Whiting, 2005
Anisolabidae	384	Labiinae + Nesogastrinae	80	Costa, 2006; Wong, Meunier and Kölliker, 2013	Deem, 2014	Jarvis, Haas and Whiting, 2005
Blattodea						
Cryptocercidae + Isoptera	3118	Blattidae	614	Costa, 2006; Hunt and Nalepa, 1994; Wong, Meunier and Kölliker, 2013	Beccaloni, 2014	Inward, Beccaloni and Eggleton, 2007
Blaberidae	1204	Ectobiidae	2426	Costa, 2006; Wong, Meunier and Kölliker, 2013	Beccaloni, 2014	Inward, Beccaloni and Eggleton, 2007
Hymenoptera						
Vespidae	710	Rhopalosomatidae	68	Hunt, 1999; Hunt and Nalepa, 1994	Lohrmann and Ohl, 2010; Richards, 1971	Branstetter et al., 2017; Hunt, 1999
Apoidea + Formicidae	34321	Scolioidea	560	Hunt and Nalepa, 1994	Ascher and Pickering, 2017	Branstetter et al., 2017
Ammophilinae	313	Sphecinae	256	Evans, 1959; Wong, Meunier and Kölliker, 2013	Pulawski, 2014	Debevec, Cardinal and Danforth, 2012

provisioning", "parental provisioning", and "regurgitation" and limited results to invertebrates, focusing mainly on the arthropods.

I classified clades as exhibiting progressive provisioning behaviour when two species within a genus exhibit it (following Pfennig and McGee, 2010). If the phylogenetic resolution of a clade was at a lower level than the genus level (i. e. subfamily, family, or order), I included the clade when two or more genera within the clade had two or more species that exhibit provisioning behaviour. Life history data were lacking for some species, which introduces uncertainty to the classifications of provisioning clades or non-provisioning clades. In addition, phylogenetic relationships between genera or families were not always present. If this was the case, I used the lowest hierarchy of clades that was available in the phylogeny (i. e. if the phylogeny was at the family level, the clades included in the analysis were of the family). Sister-clades were identified using published phylogenies, with preference given to molecular phylogenies. Morphological phylogenies based on morphological comparisons were used when molecular phylogenies could not be found. The clade exhibiting provisioning behaviour was identified on the phylogeny and its sister clade was found at the nearest node, which sometimes included more than one genus or family. If this was the case, I combined all genera or families. I searched online databases to find estimates for the number of species in each clade (see Table 7.1). Some notable examples of parental provisioning were excluded because of a lack of replication within the genus. For example, the burrower bug (*Sehirus cinctus*) provisions offspring with nutlets (Agrawal, Brodie and Brown, 2001), but remains the only species in the genus described to do so. In addition, the Jamaican bromeliad crab *Metopaulias depressus* provides resources for its offspring who develop in the water pools of bromeliads in palm trees (Diesel, 1989). It too is the only species in the genus that has currently been observed performing such a remarkable behaviour, and so these clades were excluded from this analysis.

7.4.2 *Statistical methods*

I used sister-clade comparisons to determine the correlation between progressive provisioning and species richness, as by definition sister-clades are the same age and so any difference in species richness is not attributable to the age of the clade. Though comparing sister-clades has problems (see Losos and Miles, 2002), the method has been used to good effect with respect to other traits like resource polyphenism (Pfennig and McGee, 2010), the extent of sexual selection (Barraclough, Harvey and Nee, 1995), and phytophagy (Mitter, Farrell and Wiegmann, 1988). The sister-group pairs were subjected to paired sign test and Wilcoxon matched-pairs signed rank test, as $N > 12$ (Siegel, 1956). The

sign test takes the direction of difference between sister-groups (i. e. positive or negative) and applies it against a binomial distribution. The Wilcoxon test not only takes the direction, but also the magnitude of the difference between the numbers of species in each sister group. As clades differ in the number of species in total, due to clade age or taxonomic hierarchy, the Wilcoxon test was performed on the proportion of the total number of species from each sister-clade pair in each clade, which was compared with 50% as the null expectation.

7.4.3 *Progressive provisioning is correlated with species richness*

Of the 16 sister pairs we have identified (Table 7.1), clades that exhibit parental provisioning behaviour have more species in all but three (the spider family Erisidae, the coleopteran sub-family Platypodinae, and Blaberidae cockroaches). Provisioning clades are associated with having a greater number of species than non-provisioning clades (P = 0.011), as well as the magnitude in the proportion of species (V = 126, P = 0.001, Figure 7.1).

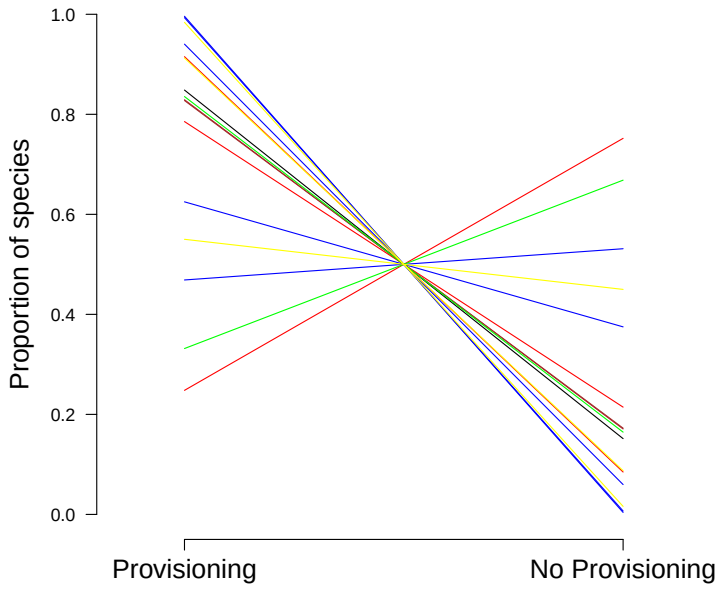


Figure 7.1 | Pairwise comparison of the number of species of sister clades that either exhibit or do not exhibit provisioning behaviour. As clades differ in the number of species in total, the data displayed are the proportion of the total number of species from each sister-clade pair in each clade. Colours indicate order: Hirudinea (black), Araneae (red), Coleoptera (blue), Dermaptera (brown), Blattodea (green), Hymenoptera (yellow).

7.5 POTENTIAL MECHANISMS

I have provided evidence linking clades that have evolved parental provisioning behaviour with greater numbers of species. I used one species to show experimentally that changes in parental care lead to rapid microevolutionary change. Here, I have shown this microevolutionary change is reflected in broad scale patterns of macroevolutionary diversification. In this chapter, I focused only on one aspect of the elaborate parental care that some organisms exhibit, namely progressive provisioning. It is not the only aspect of the family environment that can shape evolutionary trajectories, but it is certainly one of the more easily quantified aspects. I now describe the potential mechanisms that could be driving the pattern in Figure 7.1, some of which I have addressed experimentally in this thesis. All of the mechanisms below are not mutually exclusive and could apply in the evolutionary process in parallel. Many apply to progressive provisioning and can also be more broadly be applied to other aspects of parental care, and extend beyond invertebrates.

7.5.1 *The accumulation of cryptic genetic variation*

Provisioning behaviour can result in the accumulation of cryptic genetic variation, increasing genetic variation upon which adaptive evolution could work when the environment changes. With a simple single locus quantitative genetics model, Wade (1998) showed that a trait under the influence of a maternal effect will have a greater genetic variance at equilibrium, revealed as greater heterozygosity or polymorphism (Linksvayer and Wade, 2009). Differently put, genes affected by maternal effects are buffered from selection, and potential deleterious mutations arising in the offspring will be shielded from any purifying selection through the influence of the mother. One can imagine a mutation for a key enzyme, rendering it defunct for its initial role would not affect the development of the offspring if the mother partially digests the food provided for the offspring with her functional enzyme. This could potentially free the mutated enzyme to take on a different role within the offspring. Of course, most of the time the mutation will likely be deleterious, but this may also occur for neutral alleles that may confer some benefit once the environment changes. Parents can then be analogous to molecular buffering mechanisms, like heat shock proteins (Rutherford and Lindquist, 1998), which, when only mutated themselves, yield a large array of phenotypic variation derived from cryptic genetic variation that the heat shock proteins conceal.

The accumulation of cryptic genetic variation through the parental buffering process could have profound evolutionary consequences (Gibson and Dworkin,

2004; Masel, 2006; McGuigan et al., 2011; Paaby and Rockman, 2014). Standing genetic variation is thought to be the crucial fuel for local adaptation and ultimately speciation (Thompson, 2013), so it follows that any population that has a history of maternal effects has a reservoir of variation upon which adaptive evolution could act. Indeed, a new environment may provoke plastic or evolutionary changes in parental provisioning behaviour that facilitate the exposure of the cryptic variation to selective forces. This has been shown by adaptive expression of heat shock proteins in *Tribolium castaneum*, where a new stressful environment, induced by social interactions with wounded conspecifics, down regulates heat shock proteins, potentially releasing cryptic variation (Peuß et al., 2015).

Snell-Rood et al. (2016) provide a good review for the role of parental care in accumulating cryptic genetic variation as well as using two dung beetle species (*Onthophagus taurus* and *O. gazella*) to test aspects of their predictions. Dung beetles are mass provisioners that collect and store a ball of dung for a single developing larva deep underground. To induce novel mutations, Snell-Rood et al. (2016) used radiation in combination with two temperature treatments (constant or variable) to simulate variation in parental care between populations. They found that, to some degree, constant temperature may stop the deleterious mutations from manifesting during development, consistent with the idea that parents may buffer variation from exposure to selection, and therefore contribution to the accumulation of variation.

An alternative way to interpret parental care buffering cryptic genetic variation is to use inbreeding to increase homozygosity of deleterious mutations. Sociality more broadly has been thought to mitigate the ill effects of inbreeding (Avilés and Bukowski, 2006). This method was used by Pilakouta et al. (2015) with *N. vespilloides*. The effects of inbreeding for a single generation were more pronounced when parents could not care for their offspring after they hatch (Pilakouta et al., 2015), and again suggests it is highly likely that parental care can buffer mutations exposed by inbreeding from selection and thus increase cryptic genetic variation.

Further exploration of the role of parental care in accumulating genetic variation is required. In Chapter 4, I used a quantitative genetic framework to estimate cryptic genetic variation as changes in additive genetic variation across different parental care environments. In theory, additive genetic variance should increase when parents are absent because genes should then contribute more to the offspring phenotype. However, in three fitness related traits, I found no change in additive genetic variation across four environments, indicating that parents do not appear to buffer genetic variation. To get a more complete picture, further work should replicate this experiment with populations that have been

evolving without parental care. Here, one would predict greater levels of heritable variation as compared with populations that have been evolving with parental care, as selection has reduced the genetic variation present. Better yet, one could use artificial selection experiments on populations evolving with and without parental care, under the expectation that those evolving without care would have reduced genetic variation, resulting in reduced responses to selection. Mutation accumulation through continual inbreeding would therefore result in lower rates of extinction as deleterious mutations would be purged when parental care is removed. Populations that exhibit parental care, however, I would think would also have lower rates of extinction, as the build up of deleterious mutations would be buffered by parents. Figure 7.1 shows only the number of species, which could reflect changes in rates of speciation or extinction. While many of the mechanism I describe focus on speciation, they can also be applied to reducing rates of extinction and influence diversification through population persistence and allopatric speciation (see below).

7.5.2 *Range expansion*

An alternate way in which parental provisioning may facilitate diversification is through lifting constraints on range expansion. This may lead to populations encountering new environments with new selection pressures, ultimately resulting in allopatric speciation (for example, ring species Irwin et al., 2005). During the process of range expansion, the pioneering populations on the range front will be small, and therefore inbreeding is more likely to occur (Eckert, Samis and Lougheed, 2008). Parental care has been shown to buffer the deleterious effects of inbreeding, at least for one generation (Ihle, Hutter and Tschirren, 2017; Pilakouta et al., 2015). By buffering such effects, the risk of extinction is lowered, and likelihood of persistence on the range boundary increases, and this increases the possibility for adaptive evolution (Hewitt, 2000). There is also a build up of deleterious mutations as a range expands, termed the expansion load (Peischl and Excoffier, 2015; Peischl, Kirkpatrick and Excoffier, 2015; Peischl et al., 2013). The expansion load is detrimental to fitness and can limit a species' range even in a stable, constant environment (Peischl et al., 2013). Genetic variation is required at the edge of a range as a lack of variation impedes the response to selection and may slow adaptive evolution (Pujol and Pannell, 2008). As I described above, parental care can have consequences on the accumulation of genetic variation that could well facilitate range expansion through adaptive evolution.

Parents can also play a role in facilitating range expansion through their effect on dispersal dynamics. For example, maternal effects have been shown to have a profound influence on the dispersal capability of offspring, either through hatch-

ing bias, hormones deposited in eggs (Duckworth, 2009; Duckworth, Belloni and Anderson, 2015), or maternal morphology (Donohue, 1999). Social interactions between parents and offspring may serve as information about whether to disperse (Corcobado et al., 2012), and conflict arising between parents and offspring can further encourage dispersal (Cote, Clobert and Fitze, 2007).

When offspring disperse, maternal effects can potentially also contribute to success in a new niche (Fox and Savalli, 2000). In addition, parental provisioning could act as a preadaptation for the expansion in hostile environments. Social interactions in cooperatively breeding birds, for example, have been shown to facilitate colonisation of new niches once cooperation has evolved (Cornwallis et al., 2017). More generally, such a mechanism could rely on flexible parental provisioning (Royle, Russell and Wilson, 2014), allowing the adaptive plasticity to mitigate a multitude of potential challenges, like a poor resource environment (Davis, Todd and Bullock, 1999). Using a large dataset of avian parent–offspring data, Caro et al. (2016) showed that the flexibility in parent–offspring communication over provisioning of food provides a mechanism for persistence in new environments that may be poor quality, whilst maintaining the possibility of flourishing if and when the environment becomes more favourable.

Testing the causal role of parental care in range expansion is difficult. One method would be to compare the geographic ranges of closely related species or genera and ask if those that show parental care have a larger range (Pfennig and McGee, 2010). However, inferring range size retrospectively is difficult as range dynamics shift over time. An alternative is to investigate current range shifts that may be occurring through a rapidly changing climate, or an introduced species. Range expansion in the more extreme latitudes may differ because some species have parental care, or in the reverse, range shrinking may be diminished when contractions in species' ranges occur. This would provide some insight into the role of parents in ameliorating negative environmental effects, which is linked to promoting persistence and expansion into new environments.

7.5.3 *Conflict and coadaptation within the family*

Parent–offspring conflict is the mechanism that drives divergence and speciation in the VDC hypothesis (Zeh and Zeh, 2000, 2008). It could also account for the increase in species in provisioning clades. Antagonistic relationships provide the raw material with which diversification can occur through coevolutionary arms race dynamics (Dawkins and Krebs, 1979), an idea that was applied to host–parasite interactions. With Trivers' (1974) insights into family life, conflict arising between parents and their offspring can equivalently be seen as fuel for antagonistic coevolution. Imagine two populations that exhibit provisioning

behaviour. Offspring are selected to demand more than the parents are selected to provide, which may lead to the evolution of offspring traits to exploit their parents. Parents are then selected to subvert such manipulation. Due to contrasting ecological conditions of variation in average relatedness, the two different populations could therefore reach two different resolutions to parent–offspring conflict, which impose a strong post-mating barrier and ultimately speciation. When crossed, the parents from one population have not evolved to resist the manipulations of the other population's offspring, imposing a large cost on the parent. For the VDC hypothesis, this is highlighted with the invasiveness of placentation in mammals and fish (Elliot and Crespi, 2006; Schrader and Travis, 2008). The data for parent–offspring conflict driving divergence in provisioning species, however, is mixed. Conflict arising through mismatches between parental supply and offspring demand in canaries result in a reduced growth rate for offspring (Hinde, Johnstone and Kilner, 2010), which suggests that divergence can result from selection acting through parent–offspring conflict. On the other hand, Benowitz, Moody and Moore (2015) cross-fostered offspring from two species of burying beetle, *Nicrophorus vespilloides* and *N. orbicollis*, to each reciprocal parent and found no reduced fitness for the mismatches in parent and offspring. In a similar design, Linksvayer (2007) cross-fostered between three closely related *Temnothorax* ants and again found no reduced fitness when species were mismatched. These results indicate conflict may not be as important a driver of isolation in behaviourally provisioning species as it is in placental provisioning species. This conclusion probably holds true when variation in provisioning is larger within populations that between populations, with conflict over provisioning probably serving to reinforce divergence through correlated ecological shifts that indirectly influence provisioning.

However, parent–offspring conflict can, through very different means, exacerbate divergence through correlated changes in life-history traits. Comparative work on placental fish, for example, has shown that the evolution of matrotrophy drives a shift in the effect of sexual selection, accelerating the divergence between populations (Pollux et al., 2014). Using artificial selection, Kölliker et al. (2015) used earwigs (*Forficula auricularia*) to select for females based on the relative size of their second clutch to the size of their first, and in so doing manipulate the levels of parent–offspring conflict over parental investment to the second clutch. With it comes the correlated responses of development time and body size in her offspring; evolved changes in life-history traits furthering any possible divergence in another phenotypic plane, possibly unrelated to that of the conflict itself.

Sexual conflict over the provisioning of offspring could provide a different mechanism for population divergence. Sexual conflict exists over provisioning

as females invest more heavily into offspring than males (Clutton-Brock, 1991), so males are selected to exploit females to a greater extent. Sealed bid models of parental investment are often cited as the evolutionary stable strategy to maximise returns on rearing offspring (Houston and Davies, 1985). Small changes in the environment may lead to deviations from the equilibrium between populations and the roles males and females play could change and lead to reduced fitness when the two populations are drawn back together. For example, Hager and Johnstone (2003) crossed two lines of mice and found that litter size was driven by the paternal genotype whereas provisioning is driven by the maternal genotype, as a genetic manifestation of the conflict over care (Kölliker et al., 2000). Sexual conflict through provisioning can therefore lead to reduced fitness for both the parents and offspring potentially resulting in divergence (Kilner et al., 2015).

One result from parent–offspring conflict is that of coadaptation (Hinde, Johnstone and Kilner, 2010; Kölliker, Brodie and Moore, 2005; Kölliker and Richner, 2001). When parents and offspring interact, selection is acting on the parents with respect to their offspring traits, and selection is acting on offspring in relation to the traits of their parents (Godfray, 1995; Wolf and Brodie, 1998). Genes encoding, for example, begging in offspring and provisioning in parents, evolve in concert. Combinations of parent and offspring traits therefore yield greater fitness that selection on parental provisioning or offspring begging alone, where the greatest fitness for both parents and offspring is achieved through the appropriate match (Hinde, Johnstone and Kilner, 2010). The greatest fitness is therefore achieved when the begging intensity of offspring matches the provisioning ability of parents (Hinde, Johnstone and Kilner, 2010). Deviations from matches results in lower fitness, and so can act as fitness valleys if two lineages interact which have different degrees of coadaptation.

When selection acts on parents, a negative genetic correlation between parental traits and offspring traits emerges; parents that are genetically pre-disposed to be better provisioners have offspring that are genetically poor elicitors of food (Kölliker, Brodie and Moore, 2005; Wolf and Brodie, 1998). Conversely, when selection acts on offspring traits, a positive genetic correlation between parent and offspring traits is predicted, where parents who provide more food have offspring who beg more (Kölliker, Brodie and Moore, 2005; Wolf and Brodie, 1998). Negative genetic correlations exist between parents and offspring in burrower bugs (*Sehirus cinctus*, Agrawal, Brodie and Brown, 2001) and in mice (Ashbrook, Gini and Hager, 2015), and positive genetic correlations in burying beetles (*N. vespilloides*, Lock, Smiseth and Moore, 2004), great tits (*Parus major*, Kölliker et al., 2000), and a different strain of mouse (Hager and Johnstone, 2003). If different environments select on different aspects of parent–offspring interactions and create different patterns of genetic correlation between traits,

divergence between lineages would rapidly increase, furthering any barriers between populations if they meet again in sympatry.

Coadaptation, however, need not result solely from conflict. For example, eastern phoebe parents have a specific food alarm that triggers offspring begging (Madden, Kilner and Davies, 2005). This is in the best interest of both the parents, who determine when offspring beg, and the offspring, who only invest in costly begging behaviours only when necessary. Choice of oviposition site and larval performance in phytophagous insects are also coadapted, both across species (Gripenberg et al., 2010; Thompson, 1988), but also crucially, within populations of a single species (Bossart, 2003; Via, 1986). Genetic covariance for maternal host preference and larval performance exist in both the fly *Liriomyza sativae* (Via, 1986) and tiger swallowtail *Papilio glaucus* (Bossart, 2003), where mothers prefer host plants on which their offspring are best adapted. Parental host preference traits and offspring performance traits are therefore coadapted, all without the requirement of conflict. Interactions within the family offer a myriad number of mechanisms that could drive divergence in the first instance, but more likely reinforce divergence in the second instance when two previously allopatric populations again reside in sympatry.

7.5.4 *Indirect genetic effects*

When two social partners interact, the genes of the first can influence the phenotype of the second. These associative effects or indirect genetic effects (IGEs, Griffing, 1967; Wolf et al., 1998), are theorised to create feedback loops between traits in social partners that can lead to rapid evolution (McGlothlin et al., 2010; Moore, Brodie and Wolf, 1997; West-Eberhard, 1983; Wolf, Brodie and Moore, 1999) or stasis (Bijma, 2014; Bijma and Wade, 2008; Wade et al., 2010). Genes belonging to parents can therefore influence the phenotypes of their offspring above and beyond their contribution to the offspring's genome. IGEs also provide a framework by which traits without significant additive genetic variation can also evolve (Chapter 5, Jarrett et al., 2017). IGEs can work in the contexts of parent–offspring conflict and coadaptation, and may even explain patterns the other two mechanisms show. Identifying IGEs is still a difficult process, whereby using clones or close family members has yielded inferences about how genotypes in different individuals interact with one another.

7.5.5 *Symbiont inheritance*

Provisioning of offspring provides the opportunity for the vertical transfer of mutualistic symbionts. Mothers of the dung beetle *O. gazella*, leave a faecal secretion

called a pedestal, which contains the microbial contents of the maternal gut and is eaten by the larvae upon hatching (Estes et al., 2013; Schwab et al., 2016). The maternal microbiota confers benefits to larval development in environments that are known to reduce larval fitness, which increases the ability for populations to persist in novel environments (Schwab et al., 2016). An alternative route to the same goal is the evolution of allo-coprophagy in earwigs, where individuals ingest faecal matter from their siblings (Falk et al., 2014), providing a mechanism by which complex coevolution can start between host and microbiome. The gut microbiota of termites has coevolved with the termites from the early cockroach ancestor *Cryptocercus* through parental provisioning behaviour, ensuring different termite species have unique gut species and communities that may have contributed to the adaptation of the termite species (Ohkuma et al., 2009). Certainly, disrupting the microbiome of termites can have large negative effects of longevity and growth (Rosengaus et al., 2011). Vertical transmission faithfully recapitulates the parental microbiome in the offspring which could include mutualistic species (Rahman et al., 2015); vertical transmission therefore provides a mechanism that forces the co-dispersal of a mutualistic pair in which dispersal of one mutualist is hindered by the lack of dispersal of the second mutualist (Nobre, Eggleton and Aanen, 2009). Mutualists found within the microbiome should therefore be added to the list of inherited traits that pass from parent to offspring and can ultimately contribute to speciation.

7.6 CONCLUSION

The mechanisms I have outlined above are likely to work in concert and influence the degree to which other mechanisms may play a role in governing evolutionary trajectories once parental care has evolved. The greater species richness in the example clades that exhibit provisioning behaviour (Table 7.1) could be a product of a great number of the listed mechanisms, with the importance of each mechanism shifting between clades, or possibly even within clades. For example, the radiation of the termites stems from a cockroach ancestor that also provisions offspring (Inward, Beccaloni and Eggleton, 2007). A great number of studies have shown the importance of the vertically-transmitted gut flora and fauna in termite ecology, and so symbiont inheritance may have played a greater role in that radiation than another other mechanism. The evolution of the vespid wasps may have been more driven by parent–offspring coadaptation, where some wasp species lack certain enzymes, requiring parents to predigest nutrients for their developing offspring (Richards, 1971). The evolution of ambrosia beetles could be attributed both the vertical transmission of their fungal food source (Biedermann, Klepzig and Taborsky, 2009), but also as a function of

care buffering potential negative consequences of inbreeding (Jordal, Normark and Farrell, 2000). Research I have presented in this thesis suggest that in the case of the burying beetles, cooperative interactions through likely IGEs largely contributed to the radiation of *Nicrophorus* (see Chapter 5, Jarrett et al., 2017).

In this thesis, I have integrated behavioural ecology with evolutionary biology to answer a fundamental question: how does parental care shape the evolutionary process? The work I have presented has built upon research on parental effects in adaptation and divergence (Badyaev and Uller, 2009; Badyaev et al., 2002; McAdam and Boutin, 2004; Räsänen and Kruuk, 2007), but also the increasingly appreciated role of behaviour in evolution (Standen, Du and Larsson, 2014; Zuk et al., 2014). Parental behaviour has a large influence on offspring, which this thesis has shown to have more rapid, and more profound effects on the evolutionary fate of populations than previous work has indicated. What is becoming clear is that parents have the potential to affect traditional evolutionary mechanisms. Parent–offspring interactions influence dispersal patterns as well as colonisation events, changing rate of gene flow between populations. New mutations that arise can theoretically have very different effects within populations depending on the levels of parental care within the population. On range fronts, such parental buffering can have consequences for population dynamics and population sizes, changing the effects of genetic drift. Lastly, I have shown that parental care affects the way in which natural selection operates: parents alter the response of populations to selection and impose selection themselves onto the whole family.

Part V

APPENDIX

MATLAB CODE FOR MEASURING PRONOTUM SIZE

The MATLAB code I used to measure adult body size is below. I slightly adapted the code written by Santiago Herce Castañón, whom I thank immensely for his help.

```
function [] = MeasureBeetlesPronotum(InitialPhoto,
    WidthOfCoinMM)
AllFiles = dir('*_*.JPG');

NumPhotos = length(AllFiles);

Error = nan;

all_files = dir('*_*.JPG');

file_names = {all_files.name};
file_names = transpose(file_names);

length_files = length(all_files);
names = cell(1,length_files);

for i = 1:length_files
    [~, name, ext] = fileparts(file_names{i});
    name_cell = cellstr(name);
    names(1,i) = name_cell;
end

beetle_names = transpose(names);

if InitialPhoto == 0 | InitialPhoto == 1
    InitialPhoto = 1;
    BeetlesInitialMeasurements = cell(NumPhotos,1);
    BeetlesPronotumSize = nan(NumPhotos,1);
    NamePhotosDone.name = 0;

    s = get(0, 'ScreenSize');
```

```
    ReferenceFileName = 'Reference.JPG';
    ReferencePhoto = importdata(ReferenceFileName);
    ReferencePhoto(:,:,2) = 0;

    B = figure('Position', [0 0 s(3) s(4)]);

    image(ReferencePhoto);figure(gcf);
    axis image
    ReferenceValues = ginput;

    RX1 = ReferenceValues(end,1);
    RY1 = ReferenceValues(end,2);

    RX2 = ReferenceValues(end-1,1);
    RY2 = ReferenceValues(end-1,2);

    LengthRefPixels = sqrt((RX2-RX1)^2 + (RY2-RY1)^2);

    close

elseif InitialPhoto > 1
    load('BeetlesSizes')
end

s = get(0, 'ScreenSize');

try
for i = InitialPhoto:NumPhotos

    CurrentPhotoName = AllFiles(i).name;
    CurrentPhoto = importdata(CurrentPhotoName);

    B = figure('Position', [0 0 s(3) s(4)]);

    image(CurrentPhoto);figure(gcf);
    axis image

    CurrentBeetleMeasurements = ginput;
```

```
    BeetlesInitialMeasurements{i,1} =
        CurrentBeetleMeasurements;

    X1 = CurrentBeetleMeasurements(end-1,1);
    Y1 = CurrentBeetleMeasurements(end-1,2);

    X2 = CurrentBeetleMeasurements(end,1);
    Y2 = CurrentBeetleMeasurements(end,2);

    LengthPronotumPixels = sqrt((X2-X1)^2 + (Y2-Y1)^2);

    RelativeSizeBeetle = LengthPronotumPixels/LengthRefPixels
        ;

    WidthPronotumMM = RelativeSizeBeetle*WidthOfCoinMM;
    BeetlesPronotumSize(i,1) = WidthPronotumMM;

    NamePhotosDone(i).name = CurrentPhotoName;

    close

end

save ('BeetlesSizes','BeetlesPronotumSize','i', '
    BeetlesInitialMeasurements','NamePhotosDone','
    LengthRefPixels','Error', 'beetle_names')

catch

    Error = i;
    save ('BeetlesSizes','BeetlesPronotumSize','i', '
        BeetlesInitialMeasurements','NamePhotosDone','
        LengthRefPixels','Error','beetle_names')
    rethrow(lasterror)

end

end
```

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