Nutritional and social environmental effects on parental care.

Submitted by Paul Edward Hopwood, to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Biological Sciences, May 2014.

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signed: Paul E. Hopwood

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Abstract:

Parental care is likely to evolve when benefits of care are greater than costs. Provision of parental care may buffer vulnerable offspring against unpredictable or hazardous environments permitting parents to breed in situations too hostile for unassisted juvenile survival. The nature of environmental unpredictability faced by parents and their offspring (e.g., availability of nutritional resources, breeding resources and/or the strength of competition) provides the ecological context in which costs and benefits of parental traits are defined. Therefore investigations about how the environment might shape parental traits ought not only to be conducted in the laboratory but also in a natural setting where unanticipated parameters may have profound effects on theoretical predictions. I conducted a series of manipulative experiments and observational studies in the laboratory and in the field using burying beetles, *Nicrophorus vespilloides*, to examine the effects of environmental variation on parental competitive ability, reproductive productivity, longevity and the expression of parental sex-role differences and alternative reproductive tactics. In these beetles a relative size advantage confers success in contests for scarce and vital breeding resources so a central prediction was that reproductive success would be positively correlated with body size. In contrast I found that reproductive performance was favoured over contest success when nutritional resources were delayed temporarily during a developmental window. Larger beetles do win contests for breeding-resources but the benefits of being large depend on the quality of the social environment experienced (i.e., the relative size of an opponent). In a naturalistic setting, smaller males avoided direct contests because they attracted proportionately more females and as a result their breeding associations were more often monogamous. This has potential benefits for females

because they avoid female-female contests and brood parasitism. Variation in the nutritional environment provided by parents (the carcass size on which offspring are reared) directly influences body size creating a dynamism between the nutritional and social environments experienced by these beetles depending on their size, which has ramifications for their individual success and maintenance of alternative strategies in the population as a whole.

Table of Contents:

Figures and tables:

Author's declaration:

Chapter 1: benefitted from comments and corrections by Dr. Nick J. Royle. Lisa Berry assisted in wild beetle-trapping and recording presented in figure 1.3. and Emma Davey helped collect unpublished data on post-diapause breeding success.

Chapter 2: Paul E. Hopwood designed and conducted the experiments, collected the data, analysed results under the guidance of Dr. Nick J. Royle. The chapter was written for submission to *Functional Ecology* by Paul E. Hopwood with discussions, comments and corrections from Dr. Nick J. Royle and Prof. Allen J. Moore. The manuscript benefitted from pre-publishing comments and corrections from Prof. Wolf Blanckenhorn and two anonymous referees. Dr. Megan Head and Lisa Berry provided help maintaining laboratory animals and useful informal discussions. Prof. Tom Tregenza provided audio-visual equipment and technical assistance in the set-up of computer software which was used throughout these studies.

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All photographs and other graphical presentations are the author's. 'Nicrocosms' were designed and manufactured by the author.

Chapter 1, General introduction:

The overall aim of the series of experimental investigations presented in this thesis is to examine the effects of variation in the level and predictability of the nutritional and social environment on the expression of parental traits and assess downstream consequences on offspring phenotypes.

Parental care has been defined as 'any parental trait that enhances the fitness of a parent's offspring, and that is likely to have originated and/or be currently maintained for this function' (Royle, Smiseth & Kölliker 2012). Parental traits that fit this definition include non-behavioural traits (e.g., provisioning of gametes and offspring brooding) and behavioural traits (e.g., selection of oviposition sites beneficial for offspring survival and post natal food provisioning, i.e., parental behaviour *sensu* (Royle, Smiseth & Kölliker 2012)).

Variation in parental behaviour affecting the reproductive success of the parent may be selected directly to optimise the number of offspring produced over the lifetime of the parent (given the available energy necessary to produce them). Such optimisation involves the well-established theoretical trade-off between current and future reproductive expenditure (Williams 1966). Maximising offspring numbers with a finite energy budget involves another fundamental trade-off between offspring size (quality) and number (Smith & Fretwell 1974). The former problem may be resolved by fine-tuning energy demands of parental somatic maintenance and survival versus reproduction by balancing acquisition and allocation of resources among these different functions (Pianka 1976). One way to view the fitness of parents is to consider not only the lifetime number offspring produced but also the indirect returns

gained via the subsequent reproductive success of their offspring (i.e., higher quality offspring are likely to produce more grand-offspring) (Krebs & Davies 1993). Although there are potential problems with this conceptual approach (e.g., Wolf & Wade 2001), it does offer a clear framework within which interpretations of the adaptive consequences of parental traits may be discussed.

Parental traits affecting offspring traits and thus mediating these reproductive trade-offs may exert their influence through direct genetic, indirect genetic and non-genetic pathways (Moore, Brodie III & Wolf 1997; Wolf *et al.* 1998; Wolf & Wade 2009). For example a fecundity advantage conferred by large body size may be inherited directly via maternal genes for large body size. Alternatively, offspring may become relatively large via an indirect genetic effect, e.g., as a result of genetically encoded variation in rates of postnatal parental provisioning. Finally, parents may be large because the environment they experienced as juveniles influenced their size and this environment or one having similar attributes is shared by their offspring leading to a non-genetic pattern of 'inheritance' (Davis 2008; Uller 2012).

 'Parental effects' are effects that parental phenotype and/or the environment that it provides for its offspring has on the phenotype of its offspring, in excess of the contribution made by direct effects of genes inherited from parents (Moore, Brodie III & Wolf 1997; Mousseau & Fox 1998). Because the parental traits that affect offspring phenotypes may be genetically-based parental effects can be adaptively plastic, i.e., adjustment of individual parental behaviour in response to environmental variation to maximise fitness (Mousseau & Fox 1998b). These adaptive parental effects have

been described as transgenerational phenotypic plasticity (Uller 2008) because the parental response to environmental cues alters the phenotype of offspring.

In a heterogeneous environment there may be no single optimal parental strategy; reliable cues predicting the future offspring environment might enable selection for parental plasticity, producing fitness returns via more successful offspring phenotypes (Mousseau & Fox 1998a; Uller 2008; Uller 2012). When the environment is unpredictable one parental strategy might be to bet-hedge. For example, some birds 'over-produce' offspring (often with associated asynchronous hatching): a few core offspring may survive even if future rearing conditions are unfavourable (Mock & Parker 1997). Alternatively, individual plasticity might provide the means for parents to optimise performance across different environmental conditions in response to cues. Routes to fitness are very difficult to describe in an ecological context that includes resource heterogeneity, variation in frequency of competitive encounters and differences in individual mating success (Benton & Grant 2000; Kokko, Klug & Jennions 2012). Parental behaviour in an unpredictable environment should be selected to provide offspring with traits most likely to confer survival and reproductive advantages and/or minimise costs to parents of unexpected mischance.

Theoretical studies predict that interactions involving a variable physical and social environment can have important effects on individual parental behaviour (Houston 2005; Kokko & Jennions 2008). However, there are very few empirical studies that specifically examine effects of variation in social environmental and ecological factors together on parental care behaviour. Among those that have, (e.g., Leisler, Winkler & Wink 2002; Kosztolányi *et al.* 2006; AlRashidi *et al.* 2011; Westneat *et al.*

2011) studies of birds predominate (but see Brown, Morales & Summers' (2010) study of tropical frogs). Eldegard & Sonerud (2009) studied parental care decisions (desertion behaviour) and the resulting implications for offspring survival in a population of Tengmalm's owl, *Aegolius funereus*. The researchers found that wild females experiencing experimental food supplementation deserted offspring more often, and earlier (leaving their partners to care alone), than controls. Moreover, in a five-year observational study, maternal desertion rate increased with rodent abundance and deserted offspring tended to have reduced survival (Eldegard & Sonerud 2009). Thus, quantifying traits expressed by parents, and their consequences for offspring, while manipulating specific aspects of the environment(s) that individuals experience offers a means to identify phenotypic traits important to success in the variable ecological contexts in which traits evolve and are maintained.

The model organism for this series of investigations is the burying beetle *Nicrophorus vespilloides* (Fig.1.1)*, which exhibits complex parental care, some aspects of which are essential for survival of its offspring (Pukowski 1933; Eggert & Müller 1997; Scott 1998). This care includes detection, location, preparation, maintenance and defence of a small vertebrate carcass (e.g., small mammal, bird or amphibian) used as the single nutritional resource for the whole development of an entire brood. In addition parents directly provision offspring by regurgitating masticated carrion in response to begging. Both male and female parents often provide this direct postnatal care together but uniparental maternal and paternal care also occurs (Eggert & Müller 1997; Scott 1998).

Females and males search for carcasses with sensitive antennae that detect sulphur-containing volatile molecules associated with decay (Kalinová *et al.* 2009). Having located a suitable carcass, males must 'call' for a female, which they do by adopting a characteristic head-down posture while releasing species-specific pheromones (Pukowski 1933; Müller & Eggert 1987; Eggert & Müller 1989). This chemical signal may be detected and intercepted by both females and males and because carcasses of a suitable size are essential for burying beetle reproduction intrasexual fights for dominant access to a single carcass often occur (Scott 1998). Individuals with a positive size advantage are often victorious over their same-sex rivals in these contests (Otronen 1988; Lee *et al.* 2013). Nevertheless, defeated individuals usually remain for a while in the vicinity of the carcass and employ an alternative reproductive strategy (Eggert & Müller 1997). Subordinate (defeated) males attempt to secure matings as satellites with the dominant female (and/or subordinate females) while females invariably become brood-parasites and stay long enough to lay their own eggs amongst those of the dominant female (Eggert & Müller 1997). Although the brood may thus have multiple paternity (due to females being pre-mated or mating with male satellite competitors) and mixed maternity (via brood parasitic females laying their own eggs, securing dominant status greatly increases the probable proportion of parentage in the brood compared to these alternative tactics available to subordinates (Müller *et al.* 2007).

Figure 1.1: Female *Nicrophorus vespilloides* inside the 'crypt' she (and her partner) have created by burying a mouse carcass. Here she feeds her begging larvae in response to their begging behaviour.

However, males also employ another alternative mating tactic, calling without possession of a carcass, to attract females with whom to mate (Eggert & Müller 1997). This also benefits females whom having pre-mated may breed uniparentally if they later discover an unclaimed carcass (Müller *et al.* 2007).
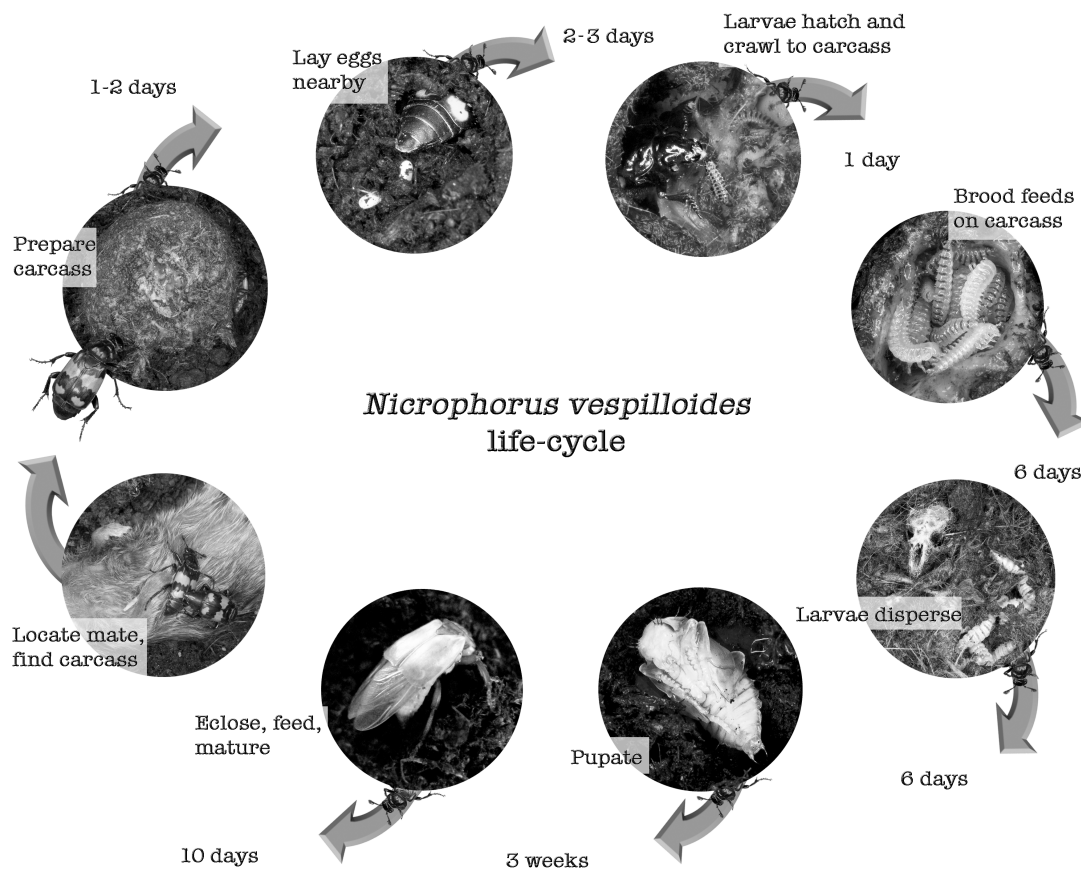
Figure 1.2: Generalised life-cycle of *Nicrophorus vespilloides* including approximate duration of each stage (repeated reproductive bouts are possible during a season and winter diapause occurs during the adult stage).

Prior to egg-laying the carcass is completely interred by beetle(s). They achieve this by continuously displacing soil substrate from beneath whilst manoeuvring the carcass by gripping it with mandibles and claws (Fabre 1918). The carcass is then stripped of fur or feathers and rolled into a neat ball reposing in a smooth-walled spherical 'crypt' a centimetre or two (sometimes more) below ground level. Bacterial and fungal invasion of the carcass is minimised by the topical application, by beetles throughout their parental attendance, of antimicrobial and antifungal oral and anal secretions. Eggs are laid over a period of approximately 30 hours and hatch asynchronously in the soil surrounding the crypt (Smiseth & Morgan 2009). Hatchling

larvae must make their own way to the carcass whereupon an incision is made by a parent through the skin of the uppermost aspect of the resource to permit larval access (Eggert & Müller 1997; Scott 1998). This incision rapidly becomes a crater through the actions of parental and larval feeding, and it expands with the growing brood into a hollow cavity within the carcass. Larvae beg for food when parents approach by rearing up and reaching out with forelegs towards a parent's mandibles (Smiseth & Moore 2002; Smiseth, Darwell & Moore 2003; Smiseth & Moore 2004; Fig. 1.1). This behaviour often extends for almost the whole of larval development on the carcass but begging and direct provisioning peak between 24 and 48 hours post-hatching (Smiseth & Moore 2002). Beyond 72 hours in ideal laboratory conditions larval growth rates in *N. vespilloides* do not appear to be affected by parental provisioning and they can achieve the same mass when parents are removed after this (Smiseth, Darwell & Moore 2003).

All somatic growth occurs as a larva on the single carcass. When the carcass is depleted of its nutrients and/or larvae have reached their final instar they disperse into the soil and pupate after a day or two (Scott 1998). Adult beetles eclose after 3 weeks pupation in the laboratory (at 20^0 C) and because this is temperature dependent (and this species over-winters in diapause as an adult) there is an extrinsic limit to the length of the reproductive season in the wild. In the laboratory however, they may be bred for multiple generations without requiring diapause. Newly eclosed beetles are not reproductively mature and must feed for approximately one week before breeding (Trumbo & Robinson 2004). Little is known about this post-eclosion feeding: carcasses can serve as a food resource and a breeding resource, but burying beetles may actively hunt invertebrates for food until

sexual maturity or when a carcass cannot be found between breeding bouts (Pukowski 1933). Females delay a further final ovarian maturation stage until a breeding carcass is located (Trumbo 1997; Scott, Carmen & Carleton 2005).

Thus the life history of burying beetles could be characterised by the juxtaposition between cycles of predictability and unpredictability they face in the wild. Finding a suitable small dead vertebrate is vital for reproductive success in females and males. Unless a male, or a female with whom he has recently mated, finds a carcass he will leave no offspring. Unpredictability of breeding resource availability may be associated with a severe, even binary fitness penalty if a carcass is not located. However, beetle offspring during development and growth experience a protected and well-provisioned nursery benefitting from the attentive ministrations of one or both parents. For larvae, even competition with siblings is smoothed via parents matching offspring number to resource mass through filial cannibalism and tailored oviposition (Bartlett 1987; Müller & Eggert 1990; Eggert & Müller 1997). In spite of this the body size of wild individuals shows marked variation (Fig. 1.3a). In 2012 I took samples from a deciduous woodland population by continuous trapping through the whole season of beetle activity measuring size (pronotum width) and weight and recording sex and number caught in 12 traps (Fig. 1.3b). The smallest individual had a pronotal width of 3.24mm and weighed 61mg and the largest was 6.07mm weighing 342mg (both males).

The data presented graphically below (Fig. 1.3b) show what appear to be two seasonal peaks of burying beetle abundance or activity. The first peak is likely to correspond with the spring emergence of adults that eclosed the previous year (this

species diapauses as an adult). The second peak is likely to comprise overlapping

generations as the first of the current year's offspring eclose. The causes of the dips

in trapped numbers, particularly at the beginning and end of August (Fig 1.3b) may

represent the influence of cool and wet weather on trapping success: beetles have to

fly to be caught and flight performance is likely to be compromised by adverse

weather conditions. Other possible explanations are that the dips correspond with

increasing proportions of beetles engaged in rearing offspring (i.e., time-out of the

mating-pool) or synchronous cyclical structure driven by the generation time of

beetles (cycles which might be maintained by recalibration each year at diapause).

Lifespan including diapause is not known in the wild but some individuals may live

for 10-15 weeks as adults in the laboratory (depending on temperature and

reproductive history) and reproduce up to five times (Ward, Cotter & Kilner 2009;

Hopwood, Moore & Royle 2013). Even when wild beetles are caught during the first

few weeks of their season (i.e., they must be last year's offspring that have spent

approximately six months in diapause) some have reproduced three times in the

laboratory (Hopwood & Davey, unpublished data).
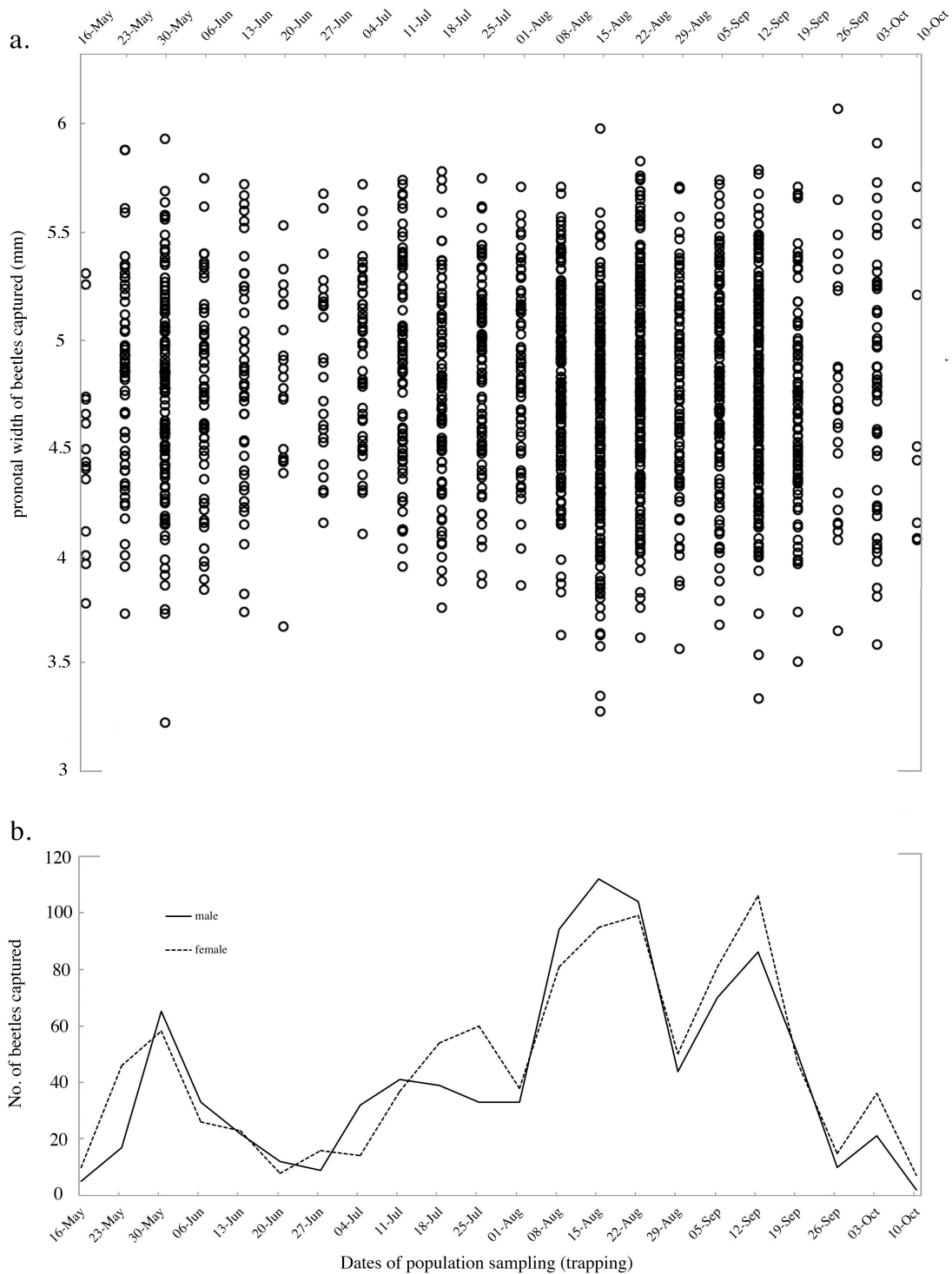
Figure 1.3: a). Body size distribution (pronotum width) of wild beetles captured continuously and collected and measured weekly, in a mixed deciduous woodland, through a single active season (2012). b). Number of beetles caught (same data as b.) in 12 funnel traps by sex. In addition a small number of beetles were captured on 2nd May (n = 4); 9th May (n = 5) and 17th Oct (n = 3) but these data are not shown.

Burying beetles thus exhibit plasticity in mating strategy and reproductive strategy; body size is extremely variable but has been established as the primary predictor of success in direct contests for essential resources (Bartlett & Ashworth 1988; Otronen 1988; Müller, Eggert & Dressel 1990; Lee *et al.* 2013). Because burying beetles exhibit female uniparental, biparental and male postnatal uniparental care and this care involves a suite of different behaviours, both indirect (e.g., preparation of carcass) and direct (i.e., regurgitating food to offspring) they are ideal subjects for studies of parental effects in the context of a heterogeneous environment. Both pre- and postnatal parental effects are important for offspring fitness (Lock, Smiseth & Moore 2004; Lock *et al.* 2007) but little is known about the role of variation in nutrition or the social environment (e.g., the intensity of competition, and the sex of competitors, for a given carcass and the resulting effects on the relatedness of the brood) in shaping parental decisions.

In controlled laboratory conditions hypotheses connected with parent/offspring conflict, resource holding potential, terminal investment, current versus future reproduction trade-offs have all been studied using burying beetles. However, there are fundamental gaps in our knowledge of how ecology shapes their evolution. Researchers to date have focussed on discrete aspects of burying beetle life history that offer the potential via laboratory controls to reduce 'environmental noise', particularly that introduced by variation in carcass size, brood size and number of competitors when interpreting experimental results. Pelletier, Garant & Hendry's (2009) neo-Dobzhanskyist rejoinder to the mantra 'nothing in biology makes sense except in the light of evolution' (Dobzhansky 1973) is 'nothing in evolution or ecology

makes sense except in the light of the other'. This thesis endeavours to synthesise and ground-truth some existing studies and to quantify some of the effects of environmental noise (i.e., variation in the nutritional environment and social/competitive environment) on parental behaviour and reproductive output.

A series of integrated manipulative studies examining burying beetles in the laboratory and in the field are presented in which nutritional and or social environments (or cues indicating these) encountered by burying beetles are manipulated at different times in their life-cycle (Fig. 1.3) to test the central hypothesis that the morphological and behavioural variation that characterises these beetles, including their notable complex parental attentiveness is a response to, and a result of environmental unpredictability. One general prediction is that where there is no single optimal strategy parents might maximise fitness by altering their behaviour or morphology in response to variation in the environment they encounter. Parental behaviour might also be targeted towards producing offspring with an appropriate phenotype for the environment they are likely to experience but when cues that predict the future environment are absent one strategy might be to bet-hedge by producing variable offspring improving the chance that a subset will be successful. In this thesis, using *N. vespilloides*, I manipulate nutritional resource availability at different life stages and introduce variation in the adult social environment (the sex and number of reproductive competitors at carcasses) in the laboratory and in the field. I present novel insights about how environmental heterogeneity shapes expression of parental traits and morphological traits and the implications of this variation for parental and offspring fitness.

In chapter 2, I examine the effect of a delay in nutrition occurring in a developmental window during which newly eclosed beetles mature sexually. Treated beetles did not differ from controls in lifetime reproductive productivity: they maintained parental performance and longevity. However they suffered reduced success in contests against size-matched rivals for breeding carcases. In chapter 3, these findings are extended with an additional manipulation of the larval nutritional environment: larvae were either reared on small or large carcasses. Contests were staged using experimental beetles, which had experienced different combinations of early nutritional treatments, against stock beetles of varying body size. Chapter 4 examines the effect of variation in the social environment experienced on parental investment. In a simulation of different sex-ratios of breeding competitors at a carcass the duration of male and female parental care and the effect on offspring were measured. Males, but not females, extended their caregiving when they faced competition from other beetles. However, extended male duration of care did not increase reproductive output or offspring fitness. Chapter 5 explores variation in the social environment further by manipulating cues to variation in the adult sex-ratio of beetles experienced by experimental males and then examining these males' responses among wild beetles in a naturalistic setting in the field. Unexpectedly, smaller males attracted a greater proportion of wild females (as first arrivals) to carcasses than did larger males potentially weakening the relationship among body size, contest success and reproductive success predicted to have been influenced by sex-ratio. In Chapter 6, having concluded in previous chapters that body size is an important trait in these beetles, I varied the size of breeding resource available to large and small pairs of beetles in the laboratory and in the field in order to determine

whether a difference in relative resource value between larger and smaller beetles would lead to individual specialisation in resource use.

References:

AlRashidi, M., Kosztolányi, A., Shobrak, M., Küpper, C. & Székely, T. (2011) Parental cooperation in an extreme hot environment: natural behaviour and experimental evidence. *Animal Behaviour, 82,* 235-243.

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

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

Benton, T.G. & Grant, A. (2000) Evolutionary fitness in ecology: comparing measures of fitness in stochastic, density-dependent environments. *Evolutionary Ecology Research, 2,* 769-789.

Brown, J.L., Morales, V. & Summers, K. (2010) A key ecological trait drove the evolution of biparental care and monogamy in an amphibian. *The American Naturalist, 175,* 436-446.

Davis, J.M. (2008) Patterns of variation in the influence of natal experience on habitat choice. *The Quarterly review of biology, 83,* 363-380.

Dobzhansky, T. (1973) Nothing in biology makes sense except in the light of evolution. *The american biology teacher, 35,* 125-129.

Eggert, A.K. & Müller, J.K. (1989) Mating success of pheromone-emitting Necrophorus males: do attracted females discriminate against resource owners? *Behaviour,* 248-257.

Eggert, A.K. & Müller, J.K. (1997) Biparental care and social evolution in burying beetles: lessons from the larder. *The evolution of social behavior in insects and arachnids* (eds J.C. Choe & B.J. Crespi), pp. 216-236. Princeton University Press, Princeton.

Eldegard, K. & Sonerud, G.A. (2009) Female offspring desertion and male-only care increase with natural and experimental increase in food abundance. *Proceedings of the Royal Society B: Biological Sciences,* **276,** 1713-1721.

Fabre, J.H. (1918) *The wonders of instinct: chapters in the psychology of insects*. T. Fisher Unwin Ltd, London.

Hopwood, P.E., Moore, A.J. & Royle, N.J. (2013) Nutrition during sexual maturation affects competitive ability but not reproductive productivity in burying beetles. *Functional Ecology,* **27,** 1350-1357.

Houston, A.I., Székely, T. & McNamara, J.M. (2005) Conflict between parents over care. *Trends in Ecology & Evolution,* **20,** 33-38.

Kalinová, B., Podskalska, H., Ruzicka, J. & Hoskovec, M. (2009) Irresistible bouquet of death—how are burying beetles (Coleoptera: Silphidae: Nicrophorus) attracted by carcasses. *Naturwissenschaften,* **96,** 889-899.

Kokko, H. & Jennions, M. (2008) Parental investment, sexual selection and sex ratios. *Journal of Evolutionary Biology,* **21,** 919-948.

Kokko, H., Klug, H. & Jennions, M.D. (2012) Unifying cornerstones of sexual selection: operational sex ratio, Bateman gradient and the scope for competitive investment. *Ecology Letters,* **15,** 1340-1351.

Kosztolányi, A., Székely, T., Cuthill, I.C., Yilmaz, K.T. & Berberoğlu, S. (2006) Ecological constraints on breeding system evolution: the influence of habitat on brood desertion in Kentish plover. *Journal of Animal Ecology,* **75,** 257-265.

Krebs, J.R. & Davies, N.B. (1993) *Behavioural ecology: an evolutionary approach,* 3rd edn. Blackwell, Oxford.

Leisler, B., Winkler, H. & Wink, M. (2002) Evolution of breeding systems in acrocephaline warblers. *The Auk,* **119,** 379-390.

Lee, V.E., Head, M.L., Carter, M.J. & Royle, N.J. (2013) Effects of age and experience on contest behaviour in the burying beetle, *Nicrophorus vespilloides*. *Behavioral Ecology,* **25,** 172-179.

Lock, J.E., Smiseth, P.T. & Moore, A.J. (2004) Selection, inheritance, and the evolution of parent-offspring interactions. *American Naturalist,* **164,** 13-24.

Lock, J.E., Smiseth, P.T., Moore, P.J. & Moore, A.J. (2007) Coadaptation of prenatal and postnatal maternal effects. *American Naturalist,* **170,** 709-718.

Mock, D.W. & Parker, G.A. (1997) *The evolution of sibling rivalry*. Oxford University Press Oxford, Oxford.

Moore, A.J., Brodie III, E.D. & Wolf, J.B. (1997) Interacting phenotypes and the evolutionary process: I. direct and indirect genetic effects of social interactions. *Evolution,* **51,** 1352-1362.

Mousseau, T.A. & Fox, C.W. (1998a) The adaptive significance of maternal effects. *Trends in Ecology & Evolution,* **13,** 403-407.

Mousseau, T.A. & Fox, C.W. (1998b) *Maternal effects as adaptations*. Oxford University Press, USA.

Müller, J.K. & Eggert, A.K. (1987) Effects of carrion-independent pheromone emission by male burying beetles (Silphidae: *Necrophorus*). *Ethology,* **76,** 297-304.

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

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

Müller, J.K., Eggert, A.K. & Dressel, J. (1990) Intraspecific brood parasitism in the burying beetle, *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Animal Behaviour,* **40,** 491-499.

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

Pelletier, F., Garant, D. & Hendry, A.P. (2009) Eco-evolutionary dynamics. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **364,** 1483-1489.

Pianka, E.R. (1976) Natural selection of optimal reproductive tactics. *American zoologist,* **16,** 775-784.

Pukowski, E. (1933) Ökologische untersuchungen an Necrophorus F. *Zoomorphology,* **27,** 518-586.

Royle, N.J., Smiseth, P.T. & Kölliker, M. (2012) *The evolution of parental care,* 1st Edition edn. Oxford University Press, Oxford.

Scott, M.P. (1998) The ecology and behavior of burying beetles. *Annual Review of Entomology,* **43,** 595-618.

Scott, M.P., Carmen, P.S. & Carleton, K.L. (2005) Quantification of vitellogenin-mRNA during maturation and breeding of a burying beetle. *Journal of Insect Physiology,* **51,** 323-331.

Smiseth, P.T., Darwell, C.T. & Moore, A.J. (2003) Partial begging: an empirical model for the early evolution of offspring signalling. *Proceedings of the Royal Society B,* **270,** 1773-1777.

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

Smiseth, P.T. & Moore, A.J. (2004) Signalling of hunger when offspring forage by both begging and self-feeding. *Animal Behaviour,* **67,** 1083-1088.

Smiseth, P.T. & Morgan, K. (2009) Asynchronous hatching in burying beetles: a test of the peak load reduction hypothesis. *Animal Behaviour,* **77,** 519-524.

Smith, C.C. & Fretwell, S.D. (1974) The optimal balance between size and number of offspring. *The American Naturalist,* **108,** 499-506.

Trumbo, S.T. (1997) Juvenile hormone-mediated reproduction in burying beetles: from behavior to physiology. *Archives of Insect Biochemistry and Physiology,* **35,** 479-490.

Trumbo, S.T. & Robinson, G.E. (2004) Nutrition, hormones and life history in burying beetles. *Journal of Insect Physiology,* **50,** 383-391.

Uller, T. (2008) Developmental plasticity and the evolution of parental effects. *Trends in Ecology & Evolution,* **23,** 432-438.

Uller, T. (2012) Parental effects in development and evolution. *The evolution of parental care* (eds N.J. Royle, P.T. Smiseth & M. Kölliker), pp. 247-266. Oxford University Press, Oxford.

Ward, R.J.S., Cotter, S.C. & Kilner, R.M. (2009) Current brood size and residual reproductive value predict offspring desertion in the burying beetle Nicrophorus vespilloides. *Behavioral Ecology*.

Westneat, D.F., Hatch, M.I., Wetzel, D.P. & Ensminger, A.L. (2011) Individual variation in parental care reaction norms: integration of personality and plasticity. *The American Naturalist,* **178,** 652-667.

Williams, G.C. (1966) Natural selection, the costs of reproduction, and a refinement of Lack's principle. *The American Naturalist,* **100,** 687-690.

Wolf, B., Brodie III, E., Cheverud, J., Moore, A. & Wade, M. (1998) Evolutionary consequences of indirect genetic effects. *Trends in Ecology & Evolution,* **13,** 64-69.

Wolf, J.B. & Wade, M.J. (2001) On the assignment of fitness to parents and offspring: whose fitness is it and when does it matter? *Journal of Evolutionary Biology,* **14,** 347-356.

Wolf, J.B. & Wade, M.J. (2009) What are maternal effects (and what are they not)? *Philosophical Transactions of the Royal Society B: Biological Sciences,* **364,** 1107-1115.

Chapter 2:

Nutrition during sexual maturation affects competitive ability but not reproductive productivity in burying beetles.

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Summary:

1. Food availability can be unpredictable. When food becomes more abundant following a period of low food availability, developing larvae or juveniles often allocate resources preferentially towards increasing growth. This has important long-term effects on adult phenotypes and longevity. Despite the importance of strategic resource allocation during early development, few studies have examined how changes in resource availability during other windows of development affect reproductive strategies and fitness independent of growth.

2. We manipulated food availability in a burying beetle, *Nicrophorus vespilloides*, during a sub-adult period of sexual maturation when individuals cannot increase structural size but are still undergoing reproductive maturation.

3. In contrast to the trade-offs during larval or juvenile growth, beetles that experienced delayed feeding during reproductive maturation allocated resources preferentially towards maintaining both reproductive function and longevity.

4. In both sexes, delayed feeding beetles were much less successful in competition for carcasses. However, delayed feeding males and females

provided similar amounts of parental care and did not differ in reproductive

success or longevity.

5. These results indicate that the nutritional environment experienced during a

key developmental window may be an important determinant of the

expression of alternative reproductive strategies in adulthood, independent of

body size.

Keywords: developmental window, life history, parental care, resource holding

potential.

Introduction:

The central tenets of life history theory involve fundamental trade-offs in the

allocation of acquired resources; firstly between somatic tissue (for development,

growth and maintenance) and reproduction, and secondly between current and

future reproductive bouts (Van Noordwijk & De Jong 1986; Roff 2002; Flatt &

Heyland 2011). The long-term consequences of such trade-offs may be notable if

resource availability is reduced or unpredictable during early growth and

development (Metcalfe & Monaghan 2001; Royle, Lindström & Metcalfe 2005; Boggs

2009). It has been hypothesized that organisms undergoing a period of nutritional

deprivation during key developmental windows may express alternative life-history

trajectories as a result of trade-offs between allocation to reproductive function and

somatic growth and maintenance (Lindström 1999; Metcalfe & Monaghan 2001;

Taborsky 2006). For many species, it is difficult to study effects occurring specifically

as a result of nutritional variation during a hypothesised developmental window

because somatic growth is often concurrent with reproductive maturation. Variation

in reproductive performance may result from adaptive resource allocation between

soma and reproduction, or correlations between growth and good condition leading to reproductive success (Blanckenhorn 2000; Gotthard, Berger & Walters 2007; Barrett *et al.* 2009), rather than as a consequence of nutritional variation during a developmental window *per se*.

The species we studied here, *Nicrophorus vespilloides* Herbst, 1783 (Fig.1.1 & Fig. 2.1), has distinct developmental stages and allows us to separate growth and reproduction. Like all holometabolous species of insects, growth during the larval stage determines (and constrains) eventual adult dimensions because body size is fixed at adult eclosion.

Figure 2.1: A pair of *N. vespilloides* on a small mammal (*Mus musculus*) carcass.

However, like many insects, an adult burying beetle must feed for several days post-eclosion to attain reproductive maturation (Pukowski 1933; Wilson & Knollenberg 1984; Trumbo, Borst & Robinson 1995). The nutritional environment experienced by post-eclosion but pre-reproductive adults during this developmental window is therefore likely to have important effects on resource allocation 'decisions' and adult phenotype independent of size.

Burying beetles (Silphidae: Nicrophorinae) exploit breeding resources (small vertebrate carcasses) characterized by unpredictable and ephemeral supply (Hanski 1990; Eggert & Müller 1997). This often leads to fierce intra-sexual competition, for carcasses suitable for breeding, among adults (Otronen 1988; Scott 1990; Scott 1994). Competitive ability is thus closely linked to reproductive outcomes through success in securing the resources required for reproduction (Müller, Eggert & Dressel 1990; Trumbo 1994), and adult body size has been found to be a major determinant of competitive success in burying beetles (Bartlett & Ashworth 1988; Otronen 1988; Müller, Eggert & Dressel 1990; Safryn & Scott 2000). However, subordinate beetles at a carcass may gain some fitness through alternative reproductive tactics. Females can resort to brood parasitic behaviour, laying a clutch of eggs nearby in the hope that some of the resulting offspring will be reared by the dominant beetle(s) (Müller, Eggert & Dressel 1990; Gross 1996; Müller *et al.* 2007). Subordinate males may adopt a satellite role, attempting to cuckold a dominant male and sneak mating with any females present (Bartlett 1988; Müller *et al.* 2007). The success of these alternative strategies is likely to be relatively low (Eggert, Otte & Müller 2008; Eggert & Müller 2011).

Burying beetle parents also have complex prenatal and postnatal parental care that influences the rate and extent of larval growth and development. Indirect care consists of parents burying a carcass, preparing and maintaining it (stripping it of fur and reducing microbe competitors), and defending it against usurpation by rivals (Walling *et al.* 2008). Direct care consists of parent beetles regurgitating masticated carrion to larvae in response to larval begging (Bartlett 1988; Walling *et al.* 2008). Broods can survive to adulthood in the laboratory without parental care, but the resulting larvae are smaller, and fewer survive compared to broods reared by one or both parents (Eggert, Reinking & Müller 1998; Smiseth, Lennox & Moore 2007).

In this study we manipulated the nutritional environment of adult *N. vespilloides* during sexual maturation to test the hypothesis that variation in resource availability, during this potential developmental window, would affect reproductive strategy independent of body size. Nutritional uncertainty might occur in nature at this stage if suitable carrion and/or invertebrate prey is not rapidly located. Our aim was to expose effects of variation in food availability during this period of sexual maturation. Specifically, we examined postnatal parental effort, and corresponding differences in fostered larvae (Experiment 1); we also tested for differences in adult competitive ability, which is important in securing a breeding resource in this species (Experiment 2). If resource allocation favours the maintenance of reproductive function then we predicted that parental effort and reproductive productivity would not differ between treated beetles and controls, but delayed feeding treatment individuals would be less successful in a contest for a breeding resource. Alternatively, if available nutritional resources are allocated preferentially to traits that increase competitive ability we predicted that beetles with delayed feeding (i.e.,

starved followed by *ad libitum* food availability) would be just as competitive as sized-matched control beetles fed *ad libitum* post-eclosion, but would have reduced reproductive productivity and/or longevity.

Methods:

Experiment 1:

Equal numbers of male and female beetles (see appendix 1 for further general methods) were allocated randomly to treatment ('delayed feeding') and control groups. Control adults (n = 20 females, n = 20 males) were weighed at eclosion then fed the standard regime in our laboratory: two decapitated mealworm larvae (*Tenebrio molitor*) twice weekly, which is an excess of food. Delayed feeding adults (n = 20 each of males and females) were weighed at eclosion and then starved for 8 days. Thereafter they were fed *ad libitum* as the controls (i.e., two mealworm larvae twice per week). Little is known about the diet of beetles in nature, specifically the extent to which they initially search for carcasses that could serve as a feeding resource and also a breeding resource, or whether they actively hunt for invertebrates until maturity. We therefore chose a period of delayed feeding as our treatment, rather than alternative feeding regimes, based on the assumption that wild beetles are likely to suffer nutritional deprivation while searching for suitable carrion, followed by potential bonanza when a carcass is found. The duration of the starvation period (8 days) was previously determined in pilot studies and showed that beetles could accommodate this level of nutritional deprivation without mortality but while losing mass. Beetles could not breed until 21 days post-eclosion to allow for any possible delay in maturation resulting from the treatment.

We let beetles rear offspring as single parents, which allowed us to examine the effect of our treatment on reproductive productivity in males as well as females and avoids confounding influences of one partner increasing parental effort to compensate for its mate. *Nicrophorus vespilloides* facultatively express all modes of parental care (biparental, uniparental female and uniparental male (Eggert & Müller 1997)). Male and female beetles provide similar care to each other when they are obliged to provide uniparental care due to a partner being removed and in this species uniparental care is as effective as biparental care (Bartlett 1988; Smiseth *et al.* 2005). Beetles from the above experiment were mated to randomly assigned opposite-sex stock individuals of similar age and breeding was initiated by placing pairs of beetles in plastic breeding boxes (17 × 11 × 5cm) with about 2cm moist soil substrate into which was placed a previously-frozen, freshly-thawed mouse carcass of a standardized size (mean ±1SE = 21.44 ± 0.11g). Stock mating-partners (male or female) were removed from breeding boxes 66 hours after eggs had been laid, the period of time during which nearly all eggs will have hatched (Smiseth, Ward & Moore 2006), so that all experimental beetles reared offspring alone (i.e., uniparental care). 'Widowed' experimental beetles were then placed individually in fresh breeding boxes, each with its own prepared carcass. Because females oviposit over several hours producing broods that hatch asynchronously these fresh breeding boxes were checked again at 72 hours for further eggs, which were then removed (this only occurred in one box containing a single female; she laid four further eggs which were removed). We used newly-hatched larvae from stock foster parents (Lock *et al.* 2007; Head *et al.* 2012) to control for variation in parental effort due to differences in brood size, propagule size or quality, or co-adaptation between parents and offspring behaviour (Lock, Smiseth & Moore 2004; Kölliker, Brodie III &

Moore 2005). In order to simulate asynchronous larval arrival, which is the natural

condition (Smiseth, Ward & Moore 2006), foster-larvae, produced by surrogate stock

beetles, were pooled on hatching and randomly allocated among experimental

single-parent breeding boxes. Five larvae were added to the soil near each foster-

carer's carcass, at intervals of 30 minutes, starting at 80 hours post-mating, until

each carer had a brood of 30 foster-larvae.

At dispersal — defined as at least two final instar larvae wandering away from the

carcass (Rauter & Moore 2002) — larvae were weighed to within 0.001g using an

electronic balance (A & D, GR-200) and experimental adults were weighed, removed

from the breeding box and housed in individual containers with fresh soil substrate

and provided with mealworms *ad libitum* as previously described.

Parental care observations:

We measured parental care in the fostered broods to evaluate postnatal reproductive

effort in both females and males. Parental care was observed 12 hours after broods

were introduced to foster-parents and repeated five times at 8 hourly intervals, to

capture the time during which parental care has the greatest impact on larval survival

and size at dispersal (Eggert, Reinking & Müller 1998; Smiseth, Darwell & Moore

2003). Breeding box lids were removed, then half an hour of undisturbed acclimation

was provided, before each set of observations was carried out under red light

(Smiseth & Moore 2004). In each of the 6 sets of observations behaviour was scored

every minute, for 30 minutes, by instantaneous sampling (Martin & Bateson 1993).

Beetles were scored as either caring or not observed caring with care including all

forms of "direct" and "indirect" care; i.e., parent observed regurgitating food to larvae

in response to begging, carcass preparation, carcass manipulation and carcass

maintenance (Walling *et al.* 2008).

Reproductive Success and Longevity:

The size (and number) of eggs laid by *N. vespilloides* may be compensated for by

postnatal care (Monteith, Andrews & Smiseth 2012). For this reason the number of

successfully dispersing larvae was used to assess reproductive success (also

allowing comparisons between uniparental males and females). In order to assess

long-term costs of the treatment, surviving adults (delayed feeding, n = 32; controls,

n = 35) were permitted two further reproductive attempts at three-weekly intervals.

Between breeding all beetles were fed excess mealworms as above. In the second

and third reproductive bouts, stock mating-partners were used with a similar

widowing process as described above. However, parents were allowed to care for

their own offspring in subsequent broods, and brood size was not experimentally

regulated in order to assess long-term reproductive productivity of beetles. All

beetles that survived the final (third) breeding bout were returned to individual boxes,

fed, and checked daily for mortality.

Experiment 2:

To test for differences in their ability to secure and hold on to resources required for

breeding (competitive ability) (Otronen 1988; Müller, Eggert & Dressel 1990), beetles

from different families were assigned to same-sex, size-matched dyads at eclosion

(n = 40), then allocated to either a control group or a delayed feeding treatment

group, as in experiment 1. Delayed feeding group beetles in each dyad were starved

for 8 days following eclosion while the control beetles were fed an excess of

mealworms 2x a week. After 8 days all beetles were weighed and fed for a further two weeks, enabling the starved treatment beetles to catch up in mass. Beetles were weighed and measured again immediately prior to contesting a carcass.

Competitive Dyads:

Contests were staged when beetles were 21 days old (post-eclosion) between dyads comprising same-sex rivals matched in size (mean width of pronotum ±1SD = 4.91 ± 0.30mm; mean of difference = 0.003 ± 0.10mm) and mass (mean ±1SD = 208.84 ± 30.51mg; mean of difference = 0.50 ± 3.54mg) that differed only in the post-eclosion feeding treatment they had received (i.e., delayed feeding vs. controls). Identification of contenders within each dyad was facilitated by marking each individual with a dot of correction-fluid either on the scutellum or the pronotum (randomized with respect to treatment between dyads). Female competitions consisted of mated females. In the wild most females finding carcasses are likely to be pre-mated, given that with a sample size of many hundreds every female we have collected from the wild has been mated (Moore, pers. obs.). We therefore mated females 24 h before contests by placing them overnight in containers with a randomly allocated stock male. A virgin stock male was introduced to a carcass 24 hours before experimental females allowing him to start preparing the resource and call. The experimental male dyad set-up was similar to the female contests except males were not already mated. For male competitions, a stock female was introduced first to the carcass and, to minimize any potential influence of female choice on the outcome of male contests, the female was pre-mated (removing her obligation to mate and reflecting the mating status of mature females we have captured in the field).

Contests were staged and filmed in the laboratory in arenas designed for use in the wild (see appendix 2). These 'Nicrocosms' allowed us to observe video footage of individual beetle interactions over the whole prenatal period when conflicts are resolved and dominance status of individuals is established. Dyads were introduced to Nicrocosms at the start of the active phase of daily cycle (early afternoon based on activity seen in the wild over 24 hours; Hopwood pers. obs.) with both rivals placed in the arena simultaneously to minimize order effects (Otronen 1988). The dominant individual in both male and female contests was defined as the beetle that secured the carcass and succeeded in becoming the carer. In the only case where both male contenders deserted, prior to larvae arriving at the carcass, the winner was determined as the male who held ground against his rival eliciting defensive behaviour and chasing him from the arena (Müller, Eggert & Dressel 1990). Each contest outcome was evaluated with the observer blind to the treatment and concluded when first instar larvae arrived at the carcass (larvae usually arrive on the fourth or fifth day).

Statistics:

All analyses were performed using 'R' version 2.14.1 (R Development Core Team 2011). Because brood size was standardized in the first breeding bout, mean mass of (foster) larvae in the first brood was used as a measure of reproductive outcomes due to variation in parental care. Reproductive success (RS) was analysed as the total production of larvae across three consecutive breeding bouts. Survival was analysed using Cox's proportional hazards and competitive ability with Fisher's exact tests (see appendices for further information and tables of results).

Results:

Experiment 1

Effect of nutritional treatment on adult mass:

At eclosion, beetles did not differ in size or mass (Fig. 2.2 and appendix 1.1, table 1) before beginning treatments or in relation to sex. Delayed feeding beetles lost 24% of their mass at eclosion by the final day of the starvation treatment eight days later, when the mean mass of the delayed feeding group was 64% the mass of controls (Fig. 2.2 and appendix 1.1, table 1). Three days after the delayed feeding group commenced feeding (i.e., on day 11) there was no longer a significant difference in mass between treatment groups (Fig. 2.2 and appendix 1.1, table 1). Parity in mass in relation to treatment and sex was maintained until breeding at 21 days post-eclosion (Fig. 2.2 and appendix 1.1, table 1).
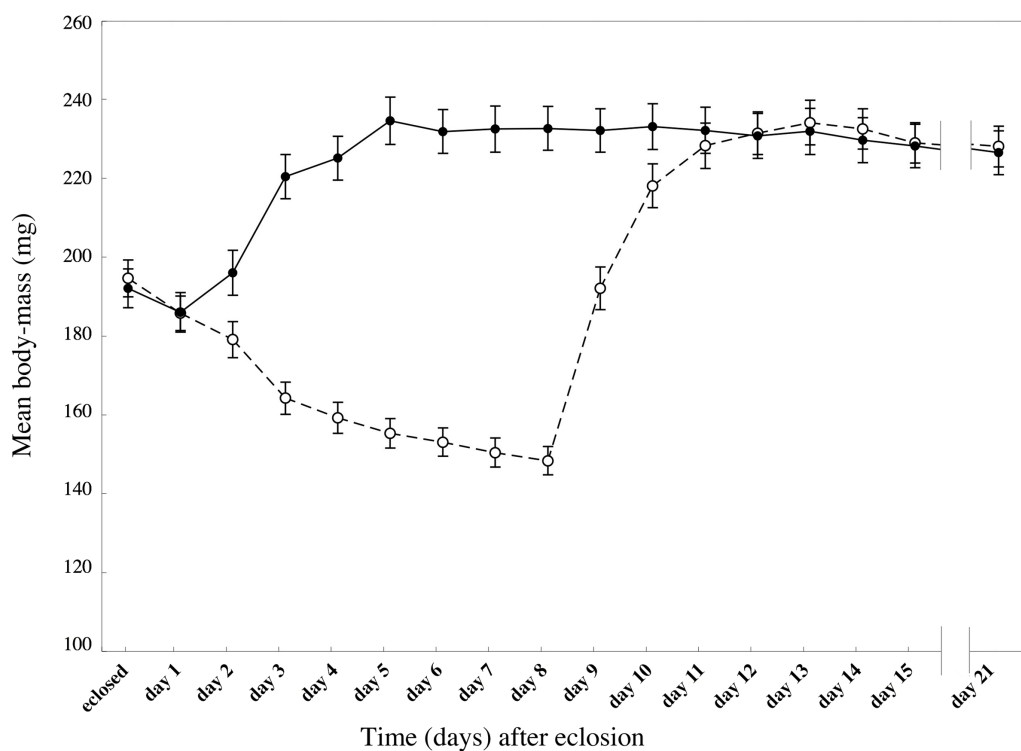

Figure 2.2: Change in daily mean mass (±1 SE) post-eclosion in control beetles (filled circles and solid line), and delayed feeding group beetles (open circles and broken line). Feeding of beetles in the delayed feeding group commenced on day 8 post-eclosion. Males and females shown combined.

Effects of nutritional treatment on parental care:

There were no statistically significant effects on proportion of time spent in direct care due to treatment (GLM with quasi-binomial errors, treatment; $F_{1,77} = 0.313$, $p = 0.577$), between males and females (sex; $F_{1,77} = 0.011$, $p = 0.919$) or interaction between sex and treatment (sex × treatment; $F_{1,74} = 1.426$, $p = 0.236$) although there was a weak positive effect of the mass of the carcass (carcass mass; $F_{1,77} = 4.051$, $p = 0.048$). There were no statistically significant effects on proportion of time spent in indirect care by treatment (treatment; $F_{1,77} = 0.308$, $p = 0.580$), sex (sex; $F_{1,77} = 2.485$, $p = 0.119$) the interaction (sex × treatment; $F_{1,74} = 2.910$, $p = 0.092$) or mass of the carcass (carcass mass; $F_{1,77} = 0.168$, $p = 0.683$).

Effects of nutritional treatment on number and size of (fostered) larvae in first brood:

In the first fostered broods a mean of 89.3% of the 30 initial larvae survived to dispersal. There was no statistically significant difference in larval survival between male and female carers: male carers mean number of larvae surviving = 26.85 ± 0.23; female carer mean number of larvae surviving = 26.77 ± 0.24, (GLM with quasi-binomial error structure, sex; $F_{1,76} = 0.033$, $p = 0.858$), nor between treatments (treatment; $F_{1,76} = 1.705$, $p = 0.196$). The interaction between sex and treatment was also non-significant: delayed feeding group mean number of larvae surviving = 26.49 ± 0.38; control mean number of larvae surviving = 27.13 ± 0.30, (sex × treatment; $F_{1,75} = 0.015$, $p = 0.903$). Larvae reared by foster-parents in the delayed feeding group had greater mean mass at dispersal than those reared by control parents: delayed feeding group mean mass = 177.33 ± 4.24mg, control mean mass = 168.46 ± 3.08mg (GLM, treatment; $F_{1,76} = 10.801$, $p < 0.002$), controlling for the significant covariate of carcass mass (carcass mass; $F_{1,76} = 11.401$, $p = 0.001$).

Effect of nutritional treatment on reproductive success (RS):

RS was calculated using the number of successfully dispersing larvae reared by beetles over all three breeding bouts (including data from individuals not surviving to rear all three broods: successful parents in bout 1, n = 79; bout 2, n = 67; bout 3, n = 47). Controlling for breeding bout (i.e., bout number included as a variable to account for the differences in larval origin among broods reared by individual parents), RS was not statistically significantly affected by treatment groups (GLMM with Poisson error structure, treatment; χ^2_1 = 0.02, p = 0.888, Fig. 2.3a), between males and females (sex; χ^2_1 = 0.757, p = 0.384), or the interaction between treatment group and sex significant (χ^2_1 = 0.143, p = 0.706).

Just over half of the beetles survived the whole experimental period and successfully reared three broods (54%; n = 47). Controlling for breeding bout, RS of these beetles was not statistically significantly different between treatment groups (GLMM with Poisson error structure, treatment; χ^2_1 = 0.099, p = 0.921), between males and females (sex; χ^2_1 = 1.815, p = 0.178) and there was no significant interaction between sex and treatment (treatment × sex; χ^2_1 = 0.436, p = 0.509).

a)

b)
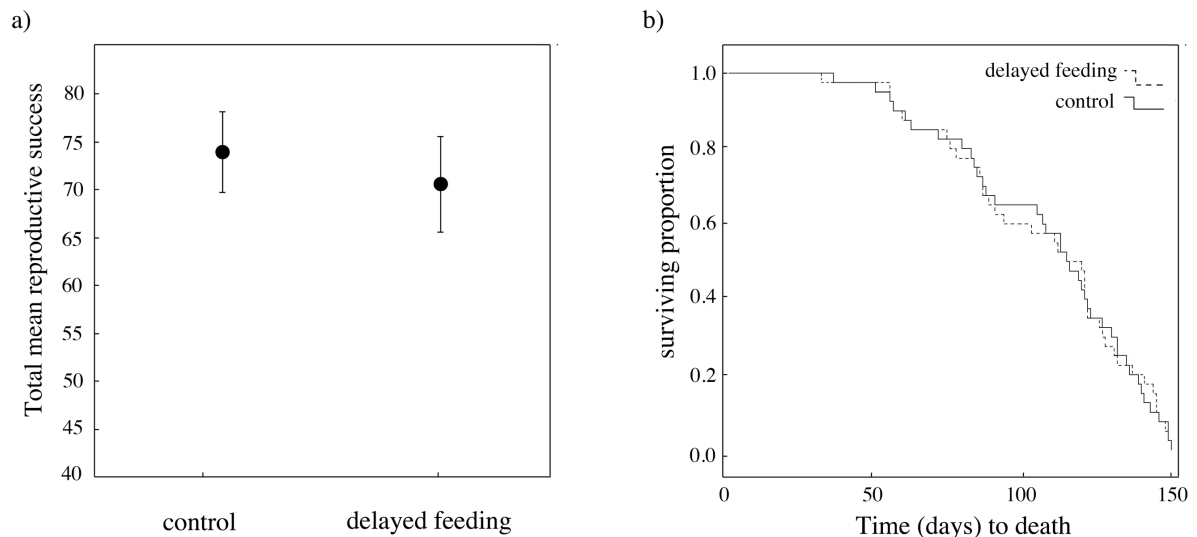
Figure 2.3: a) Total reproductive success (mean number of larvae, *per* parent, successfully reared to dispersal) between treatments over three successive reproductive bouts (including output of beetles rearing < 3 broods). Error bars = 1 SE; b) Survival curves for delayed feeding and control beetles.

Effect of nutritional treatment on longevity:

There was no statistically significant difference in survival between treatment groups: delayed feeding group mean survival (days since eclosion) = 108.23 ± 10.18 days, control group mean survival = 109.15 ± 10.01 days (Cox's proportional hazards, treatment; χ^2_1 = 0.028, *p* = 0.867, Fig. 2.3b). Males and females did not differ significantly in survival: female mean survival = 116.78 ± 8.78 days, male mean survival =100.60 ± 10.63 days (sex; χ^2_1 = 2.670, *p* = 0.102; treatment × sex; χ^2_1 = 0.766, *p* = 0.382).

Experiment 2

Effect of nutritional treatment on beetle morphometrics:

Again, beetles were not statistically significantly different in mean pronotum width or mass between treatment groups or in relation to sex at eclosion; delayed feeding

group mean pronotum width = 4.91 ± 0.05mm, control mean pronotum width = 4.92 ± 0.05mm, delayed feeding group mean mass = 182.58 ± 5.01mg, control mean mass = 182.08 ± 5.17mg (appendix 1.1, table 2). Experimental treatment groups differed significantly in mass after 8 days; delayed feeding group mean mass = 131.03 ± 3.48mg, control mean mass = 211.73 ± 5.33mg, but consistent with the first experiment, final (breeding) mass was not statistically significantly different between treatments; delayed feeding group mean mass = 209.88 ± 4.72mg, control mean mass = 207.8 ± 4.98mg (appendix 1.1, table 2).

Effect of nutritional treatment on competitive ability:

The dietary treatment had a significant effect on the winners of contests; delayed feeding individuals lost 37 out of 40 trials (Fisher's exact probabilities test, $p < 0.0001$, $n = 40$). Outcomes of contests with respect to treatment did not differ between the sexes—females in delayed feeding treatments won contests twice out of twenty trials; a single delayed feeding male won a contest out of twenty trials (Fisher's exact probabilities test, $p > 0.9$, $n = 40$).

Discussion:

Our results indicate that variation in resource availability during a key developmental window (i.e., sexual maturation of post-eclosion beetles) resulted in preferential allocation to the maintenance of reproductive potential and not competitive ability, independent of body size, in *N. vespilloides*. Delayed feeding males and females contesting a breeding resource against beetles of the same sex, similar age, size and mass at eclosion, were far less likely to secure dominant reproductive roles on the mouse carcass than beetles fed *ad libitum*. However, if given the opportunity to

breed in the absence of competition, delayed feeding beetles produced heavier larvae yet had similar RS and longevity across three successive bouts of breeding compared to controls.

This resource allocation strategy is likely to have significant effects on the reproductive success of individuals in the wild. In similar experiments, defeated males and females have never been observed in successful carcass takeovers after a dominant pair are tending larvae, but defeated males are frequently seen sneaking copulations with the dominant female and defeated females mate with the dominant male (Hopwood pers. obs.). Mating tactics of male and female beetles in nature have been shown to depend on, and change in response to, the level of competition and the individual's dominance status at a carcass; subordinate beetles almost invariably adopt brood parasitic or satellite roles (Müller *et al.* 2007). Therefore, variation in nutritional availability affecting competitive ability might be an important determinant of subsequent reproductive strategies.

In our experiment, contests were rapidly resolved with no subsequent reversals witnessed, and same-sex delayed feeding rivals almost always became subordinate. In *N. vespilloides* dominance status at a carcass, of both sexes, is crucial to individual relative reproductive success. Müller *et al.* (2007) found that fewer than half of carcasses they experimentally placed in the wild were uncontested by *N. vespilloides* females. Similarly, there was more than one male present on 53% of carcasses where pre-mated females did not breed uniparentally. After assigning parentage to the larvae resulting from these broods, Müller *et al.* (2007) found that male and female dominant parents produced significantly more offspring than

satellites or parasites that used the same carcasses. The dominant female beetle may actively reduce the fitness of subordinate rivals by preventing them feeding (on the carcass), thereby limiting their energetic income for producing brood parasitic egg clutches (Eggert, Otte & Müller 2008). She may also cannibalize larvae that hatch outside the expected time of her own (Eggert & Müller 2011). A dominant male can increase his fitness by repeatedly mating with the dominant female (and with any other females that arrive) thus improving his proportion of brood paternity (House, Hunt & Moore 2007). Moreover, a dominant parent can maintain its own condition through feeding on the carcass, while actively excluding subdominant competitors from the opportunity to feed. Thus, the likelihood of individual success in future breeding attempts may be affected by nutritionally-mediated dominance status at a carcass. Walling *et al*. (2009) showed that adult social experience does not affect reproductive behaviour but the nutritional environment experienced as larvae affects body size and, therefore, competitive ability. The current study shows that the nutritional environment experienced during sexual maturation also affects competitive ability in *N. vespilloides*; it demonstrates the importance of timing of variation in the availability of resources on fitness.

Because we manipulated food availability during a known reproductive developmental window (Trumbo & Robinson 2004), we predicted that nutritional stress would lead to differences in strategic allocation of resources to reproduction. However, the early post-eclosion nutritional environment experienced by beetles appeared to have little effect on parental effort of beetles. Male and female beetles that experienced the delayed feeding treatment maintained a similar level and pattern of care, with respect to the components of care, to control beetles. Levels of

uniparental care provided by male and female beetles were similar (Bartlett 1988; Smiseth *et al.* 2005). That the delayed feeding treatment did not unduly compromise quality of care is supported by the greater mean mass of the foster larvae raised by delayed feeding treatment beetles than those cared for by control parents. One explanation for this finding might be terminal investment. The terminal investment hypothesis predicts that as the probability of further reproductive opportunities declines individuals should invest more energy in the current reproductive event discounting potential benefits from future reproduction (Williams 1966, Clutton-Brock 1984). Therefore, when presented with a breeding opportunity, a beetle in sub-optimal condition should invest relatively heavily in the current brood at the expense of (relatively unlikely) future reproductive success. We were unable to determine clear relationships between our metrics of parental effort and the resulting mass of larvae. However, it is possible that transgenerational parental effects may confer indirect fitness benefits for parents. For example, increasing current reproductive effort to produce offspring endowed with increased relative competitive prowess might be a good strategy when resources are scarce or the mean body size of individuals in the population is small. In addition, in nature not all carcasses are located by multiple beetles (Müller *et al.* 2007) and a poor nutritional start in life might be mitigated by active avoidance of contested carcasses. These possibilities remain to be explored.

Studies across various taxa have shown trade-offs between allocations of resources to reproduction versus soma following periods of poor early nutrition (Blount *et al.* 2006; Barrett *et al.* 2009; Kubička & Kratochvíl 2009; Auer *et al.* 2010). We therefore looked for latent fecundity or survival costs incurred by our delayed feeding

treatment in the two subsequent breeding bouts. However, delayed feeding group beetles showed no detectable reduction in reproductive productivity or decrease in survival relative to controls (Fig. 2.3). Mortality affected mean reproductive output through time; only 43 individuals (53.75%) successfully produced a third brood, but there was no difference in mortality between treatment groups. In the relatively benign laboratory environment, nutritionally compromised individuals did not have to risk injury in contests for mates, or breeding resources, or expend energy in searching for resources, calling for mates, or defending carcasses; all of which may be important in the wild. Nonetheless, almost half of the beetles did not survive to complete three breeding attempts, increasing our confidence that we would have found differences between treatment groups had they been substantive. In our study there was no evidence for costs to reproductive productivity or longevity of the early delayed feeding (if anything there was evidence of positive, short-term, benefits to reproductive success), but costs were paid through substantially reduced ability to compete for access to carcasses.

Conclusions:

Different timing of food limitation has different effects. Our study underlines the importance of resource availability, during a key window of development, on the competitive ability, and thus resource holding potential, of burying beetles. Similar effects of nutritional variation during developmental windows may exist cryptically in other species. Most studies of food restriction have focused on early development and found a trade-off in fecundity and longevity; competitive ability is rarely addressed. Reduced competitive ability may have consequences that persist through the lifespan of individuals. Selection may therefore favour facultative adjustment of

reproductive behaviour if nutritional history acts as a cue to likely future reproductive success (e.g., the extent of resource availability or probability of success in contests). In burying beetles reduced competitive performance, mediated by their nutritional history, may lead to expression and maintenance of alternative reproductive tactics. The timing and effects of environmental sources of variation in strategies during reproduction may help explain the maintenance of variation in fitness-related traits in burying beetles and other species.

References:

Auer, S.K., Arendt, J.D., Chandramouli, R. & Reznick, D.N. (2010) Juvenile compensatory growth has negative consequences for reproduction in Trinidadian guppies (*Poecilia reticulata*). *Ecology Letters,* **13,** 998-1007.

Barrett, E.L.B., Hunt, J., Moore, A.J. & Moore, P.J. (2009) Separate and combined effects of nutrition during juvenile and sexual development on female life-history trajectories: the thrifty phenotype in a cockroach. *Proceedings of the Royal Society B,* **276,** 3257-3264.

Bartlett, J. (1988) Male mating success and paternal care in *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Behavioral Ecology and Sociobiology,* **23,** 297-303.

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

Blanckenhorn, W.U. (2000) The evolution of body size: what keeps organisms small? *Quarterly Review of Biology***,** 385-407.

Blount, J.D., Metcalfe, N.B., Arnold, K.E., Surai, P.F. & Monaghan, P. (2006) Effects of neonatal nutrition on adult reproduction in a passerine bird. *Ibis,* **148,** 509-514.

Boggs, C.L. (2009) Understanding insect life histories and senescence through a resource allocation lens. *Functional Ecology,* **23,** 27-37.

Eggert, A.K. & Müller, J.K. (1997) Biparental care and social evolution in burying beetles: lessons from the larder. *The evolution of social behavior in insects and arachnids* (ed. J.C.C. Choe, B.J.), pp. 216-236. Princeton University Press, Princeton.

Eggert, A.K. & Müller, J.K. (2011) Timing of oviposition enables dominant female burying beetles to destroy brood-parasitic young. *Animal Behaviour,* **82,** 1127-1233.

Eggert, A.K., Otte, T. & Müller, J.K. (2008) Starving the competition: a proximate cause of reproductive skew in burying beetles (*Nicrophorus vespilloides*). *Proceedings of the Royal Society B,* **275,** 2521-2528.

Eggert, A.K., Reinking, M. & Müller, J.K. (1998) Parental care improves offspring survival and growth in burying beetles. *Animal Behaviour,* **55,** 97-107.

Flatt, T. & Heyland, A. (2011) *Mechanisms of Life History Evolution: The Genetics and Physiology of Life History Traits and Trade-Offs*. Oxford University Press, Oxford.

Gotthard, K., Berger, D. & Walters, R. (2007) What keeps insects small? Time limitation during oviposition reduces the fecundity benefit of female size in a butterfly. *The American Naturalist,* **169,** 768-779.

Gross, M.R. (1996) Alternative reproductive strategies and tactics: diversity within sexes. *Trends in Ecology & Evolution,* **11,** 92-98.

Hanski, I. (1990) Dung and carrion insects. *Living in a patchy environment* (eds B. Shorrocks & I.R. Swingland), pp. 127-145. Oxford University Press, Oxford.

Head, M.L., Berry, L.K., Royle, N.J. & Moore, A.J. (2012) Paternal Care: Direct and indirect genetic effects of fathers on offspring performance. *Evolution,* **66,** 3570-3581.

House, C.M., Hunt, J. & Moore, A.J. (2007) Sperm competition, alternative mating tactics and context-dependent fertilization success in the burying beetle, *Nicrophorus vespilloides*. *Proceedings of the Royal Society B,* **274,** 1309-1315.

Kölliker, M., Brodie III, E.D. & Moore, A.J. (2005) The coadaptation of parental supply and offspring demand. *American Naturalist,* **166,** 506-516.

Kubička, L. & Kratochvíl, L. (2009) First grow, then breed and finally get fat: hierarchical allocation to life-history traits in a lizard with invariant clutch size. *Functional Ecology,* **23,** 595-601.

Lindström, J. (1999) Early development and fitness in birds and mammals. *Trends in Ecology & Evolution,* **14,** 343-348.

Lock, J.E., Smiseth, P.T. & Moore, A.J. (2004) Selection, inheritance, and the evolution of parent-offspring interactions. *American Naturalist,* **164,** 13-24.

Lock, J.E., Smiseth, P.T., Moore, P.J. & Moore, A.J. (2007) Coadaptation of prenatal and postnatal maternal effects. *American Naturalist,* **170,** 709-718.

Martin, P.R. & Bateson, P. (1993) *Measuring behaviour: an introductory guide*. Cambridge University Press.

Metcalfe, N.B. & Monaghan, P. (2001) Compensation for a bad start: grow now, pay later? *Trends in Ecology & Evolution,* **16,** 254-260.

Monteith, K., Andrews, C. & Smiseth, P. (2012) Post-hatching parental care masks the effects of egg size on offspring fitness: a removal experiment on burying beetles. *Journal of Evolutionary Biology*.

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

Müller, J.K., Eggert, A.K. & Dressel, J. (1990) Intraspecific brood parasitism in the burying beetle, *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Animal Behaviour,* **40,** 491-499.

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

Pukowski, E. (1933) Ökologische untersuchungen an Necrophorus F. *Zoomorphology,* **27,** 518-586.

R Development Core Team (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org.

Rauter, C. & Moore, A. (2002) Evolutionary importance of parental care performance, food resources, and direct and indirect genetic effects in a burying beetle. *Journal of Evolutionary Biology,* **15,** 407-417.

Roff, D. (2002) *Life History Evolution*. Sinauer Associates, Massachusetts.

Royle, N.J., Lindström, J. & Metcalfe, N.B. (2005) A poor start in life negatively affects dominance status in adulthood independent of body size in green swordtails *Xiphophorus helleri. Proceedings of the Royal Society B: Biological Sciences,* **272,** 1917-1922.

Rozen, D., Engelmoer, D. & Smiseth, P. (2008) Antimicrobial strategies in burying beetles breeding on carrion. *Proceedings of the National Academy of Sciences,* **105,** 17890-17895.

Safryn, S.A. & Scott, M.P. (2000) Sizing up the competition: Do burying beetles weigh or measure their opponents? *Journal of Insect Behavior,* **13,** 291-297.

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

Scott, M.P. (1994) The benefit of paternal assistance in intra-and inter-specific competition for the burying beetle, *Nicrophorus defodiens*. *Ethology Ecology and Evolution,* **6,** 537-543.

Smiseth, P., Ward, R. & Moore, A. (2006) Asynchronous hatching in *Nicrophorus vespilloides*, an insect in which parents provide food for their offspring. *Functional Ecology,* **20,** 151-156.

Smiseth, P.T., Darwell, C.T. & Moore, A.J. (2003) Partial begging: an empirical model for the early evolution of offspring signalling. *Proceedings of the Royal Society B,* **270,** 1773-1777.

Smiseth, P.T., Dawson, C., Varley, E. & Moore, A.J. (2005) How do caring parents respond to mate loss? Differential response by males and females. *Animal Behaviour,* **69,** 551-559.

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

Smiseth, P.T. & Moore, A.J. (2004) Signalling of hunger when offspring forage by both begging and self-feeding. *Animal Behaviour,* **67,** 1083-1088.

Taborsky, B. (2006) The influence of juvenile and adult environments on life-history trajectories. *Proceedings of the Royal Society B,* **273,** 741-750.

Trumbo, S.T. (1994) Interspecific competition, brood parasitism, and the evolution of biparental cooperation in burying beetles. *Oikos,* **69,** 241-249.

Trumbo, S.T., Borst, D.W. & Robinson, G.E. (1995) Rapid elevation of juvenile hormone titer during behavioral assessment of the breeding resource by the burying beetle, *Nicrophorus orbicollis*. *Journal of Insect Physiology,* **41,** 535-543.

Trumbo, S.T. & Robinson, G.E. (2004) Nutrition, hormones and life history in burying beetles. *Journal of Insect Physiology,* **50,** 383-391.

Van Noordwijk, A. & De Jong, G. (1986) Acquisition and allocation of resources: their influence on variation in life history tactics. *American Naturalist,* **128,** 137-142.

Walling, C.A., Stamper, C.E., Salisbury, C.L. & Moore, A.J. (2009) Experience does not alter alternative mating tactics in the burying beetle *Nicrophorus vespilloides*. *Behavioral Ecology,* **20,** 153-159.

Walling, C.A., Stamper, C.E., Smiseth, P.T. & Moore, A.J. (2008) The quantitative genetics of sex differences in parenting. *Proceedings of the National Academy of Sciences USA,* **105,** 18430-18435.

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

Chapter 3:

Effects of resource variation during early life and adult social environment on contest outcomes in burying beetles: silver spoon or context-dependent strategy?

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Summary:

Good early nutritional conditions may confer a lasting fitness advantage over individuals suffering poor early conditions (a 'silver spoon' effect). However, an individual might maximise fitness if it can prepare for the future by responding to cues provided by poor developmental conditions that accurately predict a poor adult environment (environmental-matching effect). Here we test for silver spoon and environmental-matching effects by manipulating the early nutritional environment of *Nicrophorus vespilloides* burying beetles. We manipulated nutrition during two specific early developmental windows: the larval environment and the post-eclosion environment. We then tested contest success in relation to variation in adult social environmental quality experienced (defined according to whether contest opponents were smaller (good environment) or larger (poor environment) than the focal

individual). Variation in the larval environment influenced adult body size but not contest success *per se* for a given adult social environment experienced (an 'indirect' silver spoon effect in that there is no intrinsic individual disadvantage attributable to the developmental environment, but there might be more likelihood of experiencing an unfavourable adult social environment). Variation in post-eclosion environment affected contest success dependent on the quality of the adult environment experienced (a context-dependent 'direct' silver spoon effect). In contrast, there was no evidence for environmental-matching. The results demonstrate the importance of social environmental context in determining how variation in nutrition in early life affects success as an adult.

Keywords: silver spoon, environmental-matching, social environment, resource holding potential, developmental effects.

Introduction:

Variation in nutrition experienced by individuals during development can have long-term effects on adult phenotype such as body mass (Barrett, Moore & Moore 2009), fecundity (Dmitriew & Rowe 2011), dominance status (Royle, Lindström & Metcalfe 2005) and longevity (Birkhead, Fletcher & Pellatt 1999) that directly affect the fitness of individuals. Furthermore, early-life developmental effects on phenotypes may also impact on the expression of traits in other individuals (Moore, Brodie III & Wolf 1997) and even influence the population dynamics and evolutionary trajectories of organisms (Benton *et al.* 2005; Uller 2012).

Nutritional variation during early development is hypothesised to affect fitness in different ways depending on the quality of the subsequent adult environment

experienced (Grafen 1988; Monaghan 2008). For example, an advantage in adulthood for individuals with plentiful early developmental resources over those that experienced poorer early conditions, regardless of their adult environment, is known as a 'silver spoon' effect (Grafen 1988; Monaghan 2008). Alternatively, the 'environmental-matching' hypothesis predicts individuals whose adult environment 'matches' that which they experienced during development will have highest fitness (Monaghan 2008). In environmental-matching, phenotypic attributes expressed as a result of poor developmental conditions are purported to 'program' an individual to deal with correspondingly poor conditions in adulthood such that in these circumstances they even outperform individuals that experienced better developmental conditions (Gluckman, Hanson & Spencer 2005; Monaghan 2008). While there is empirical support for silver spoon effects in general (Blount *et al.* 2006; Taborsky 2006; Van De Pol *et al.* 2006; Dmitriew & Rowe 2011; Krause & Naguib 2014) and even environmental 'mismatching', with performance disproportionately bad when both developmental and adult environments are poor (e.g., Barrett *et al.* 2009) there is little or no clear empirical support for the environmental-matching hypothesis (but see Saastamoinen *et al.* 2010; Butler & McGraw 2012).

Lack of support for environmental-matching may reflect the fact that in nature the quality of the environment that individuals experience is likely to be primarily determined by their competitive ability in relation to the competitive ability of other individuals in the population (i.e., the social environment), rather than resource abundance *per se* (i.e., the physical environment). Variation in the abundance of food, for example, may not affect all members of the population equally if individuals also vary in their competitive ability, which determines their access to such

resources. In addition the particular social environment experienced by individuals (e.g., the sex and/or competitive ability of conspecifics) can also impact the expression of traits (Moore, Brodie III & Wolf 1997), and developmental conditions themselves may evolve through changes in the population social environment (Benton *et al.* 2005). However, overall levels of competition for resources will be affected by the abundance of those resources, so there is considerable feedback between the physical environment and the social environment that influences the quality of environments that individuals experience. Despite the importance of social context in determining the quality of adult environments a recent review by Monaghan (2008) showed that the majority of studies that test the effects of variation in resource availability early in life on subsequent adult traits define adult environment quality in terms of food abundance and do not consider the social environment.

In addition to not measuring environmental quality in an appropriate context another reason why environmental-matching may be poorly supported is because the early developmental environment is often loosely defined, including any or all of the period between conception and developmental maturity; an approach that implicitly assumes that effects of variation in nutrition on adult phenotype will be largely independent of when they occur during development. Recent studies have demonstrated the importance of the timing of nutritional deprivation on success in adulthood (Hopwood, Moore & Royle 2013; Saeki & Crowley 2013), which may have independent or interactive effects on the expression of adult phenotypes. Distinct stages may often exist in the developmental processes of organisms when nutritional variation has disproportionately large effects on phenotypic expression (Gilbert

2005), including effects on disease susceptibility in later life (Waterland & Jirtle 2004) and adaptive polyphenisms, such as the winged phase switch in pea aphids, *Acyrthosiphon pisum* (Brisson & Stern 2006) or horn elongation in dung beetles, e.g., *Onthophagus taurus* and *O. acuminatus* (Emlen 2000; Nijhout 2003). These 'developmental windows', although potentially important, can be difficult to identify because developmental progression may involve multiple critical windows with interacting effects on the expression of phenotypes (Nussey *et al.* 2007; Segers & Taborsky 2011). The impact of variation in nutrition early in life versus later in life may also be hard to quantify because many species experience fluctuations in availability of nutrition throughout postnatal, juvenile and adult development. For example, in many seabirds pre-reproductive development may extend over several years including prenatal, post-hatching, pre-independent and juvenile (pre-reproductive) stages (Spear & Nur 1994) and early experience may be correlated with later experience due to carry-over and cohort effects (Van De Pol *et al.* 2006; Descamps *et al.* 2008).

We previously identified a key developmental window in the burying beetle *Nicrophorus vespilloides* during which variation in food availability determined later success as an adult in contests for breeding resources (Hopwood, Moore & Royle 2013). This developmental window occurs during the period when beetles are undergoing reproductive maturation, and may last less than a week from the time the mouthparts of an eclosed beetle have sclerotized and feeding commences (~36 hours post-eclosion) until viable matings can take place (Hopwood, Moore & Royle 2013, and PEH 2013, unpublished data). Burying beetles feed on putrescent carrion and various invertebrates so variation in food availability in the wild is likely to occur

due to rainfall and temperature fluctuations affecting beetle (and potential prey) activity, and through stochastic availability of carrion. However, this post-eclosion nutritional bottleneck is not the only window during which variation in nutrition may have long-term effects on adult traits; the social and nutritional environment experienced during larval development is also important (Bartlett & Ashworth 1988).

In burying beetles the impact of the adult social environment on individual fitness is particularly important because a fundamental aspect of their life-cycle involves direct contests for breeding resources (vertebrate carcasses). Success in contests over suitable breeding resources in *N. vespilloides* is closely related to reproductive success in both sexes (Müller, Eggert & Dressel 1990). A single small carcass (e.g., mice, shrews, small songbirds) is the sole resource necessary to rear a brood of offspring. Males that locate a carcass but are then unable successfully to become the dominant male may adopt a subordinate strategy and sneak copulations with females; likewise any female unable to secure the dominant female position may parasitize a brood by laying eggs nearby (Müller, Eggert & Dressel 1990). However, parentage proportion in broods is lower for subordinates compared to the dominant pair (Müller *et al.* 2007). Thus, from an individual's perspective, its own relative size among competitors at a carcass characterises the quality of the reproductive environment (Otronen 1988; Lee *et al.* 2013). Moreover, it is possible that the body size of an individual (related to the quality of its developmental environment), might itself act as an intrinsic cue of social environmental quality, i.e., whether it is likely to be larger or smaller than competitors. For example, experimental evidence in a congener, *N. orbicollis,* suggests that small males may preferentially employ an alternative reproductive tactic spending proportionally more time trying to call

females with pheromones (with whom they mate in the absence of a carcass) than do large males (Beeler, Rauter & Moore 1999).

In the current study we test for silver spoon and environmental-matching effects by manipulating the early nutritional environment of *N. vespilloides*. In contrast to most previous studies that treat early development as a single, largely homogeneous juvenile stage, we independently manipulated larval and post-eclosion pre-reproductive nutritional windows. This is because good nutrition during the first (larval) window, if considered alone, might produce relatively large adults endowed with a putative silver spoon competitive advantage in proportion to their relative size, independent of later environmental conditions. Similarly, if relative size was controlled for and variation in post eclosion nutrition considered alone, any advantage might also suggest a silver spoon effect. However, potential interactions between these developmental windows would be missing, e.g., smaller individuals might bear food shortage better than larger individuals. Furthermore, contests only take place in the presence of a large enough carcass on which to breed, so variation in the quality of the environment is primarily determined by variation in the social environment in which any contests take place (i.e., relative competitive ability of individuals). Variation in the relative size of competing individuals therefore represents variation in the quality of the adult environment experienced by focal individuals. We produced four different early-environment treatments: (GG = control: standardized 'good' laboratory conditions throughout development (reared on a large carcass as larvae and *ad libitum* food post-eclosion); GP = good larval environment (large carcass) + poor (delayed-feeding) post-eclosion environment; PG = poor larval environment (small carcass) + good post-eclosion environment (i.e., *ad libitum*

feeding immediately from eclosion); PP = poor larval environment + poor post-eclosion environment). This allowed us to quantify the effects of different combinations of dietary manipulation treatments on adult competitive ability in *N. vespilloides* (i.e., success in competition for carcasses) for both males and females. It is known that variation in larval nutrition has permanent effects on an individual's body size (Bartlett & Ashworth 1988), and that larger individuals are more successful in contests for carcasses than smaller beetles (Bartlett & Ashworth 1988; Otronen 1988), so we predicted a positive relationship between the relative size of a focal individual compared to its opponent and the probability of contest success (Fig. 3.1a). Thus, the adult (social) environment was defined as good when focal beetles were larger than their opponent, or poor when focal beetles were smaller than their opponent. Competitors for carcasses vary in number and size in the wild (Müller *et al.* 2007), so the probability of encountering a 'good' contest environment is lower for relatively small beetles.

A clear silver spoon effect would be supported if individuals that have experienced good larval and/or good post-eclosion treatments always do better than individuals that experienced poor larval and/or poor post-eclosion treatments for any given environment experienced in adulthood (i.e., will have higher elevation relationship between the probability of winning and body size difference - see Fig. 3.1a). For example, if there is a silver spoon effect of post-eclosion delayed-feeding we would expect to see animals in GG & PG treatment groups having greater probability of success in contests than those in GP & PP groups, and if there is a silver spoon effect of a good larval treatment GG & GP will have higher probability of success

across social environments compared to beetles in PG & PP treatments (e.g., Fig. 3.1b).

In contrast, environmental-matching would be supported if there is a significant interaction between one or both of the nutritional treatments and the adult environment experienced, i.e., individuals that experienced poor early-life environments should have greater probability of success than beetles that had good early-life environments when the adult environment they experience is poor (i.e., when they are smaller than their opponent; Fig. 3.1c).
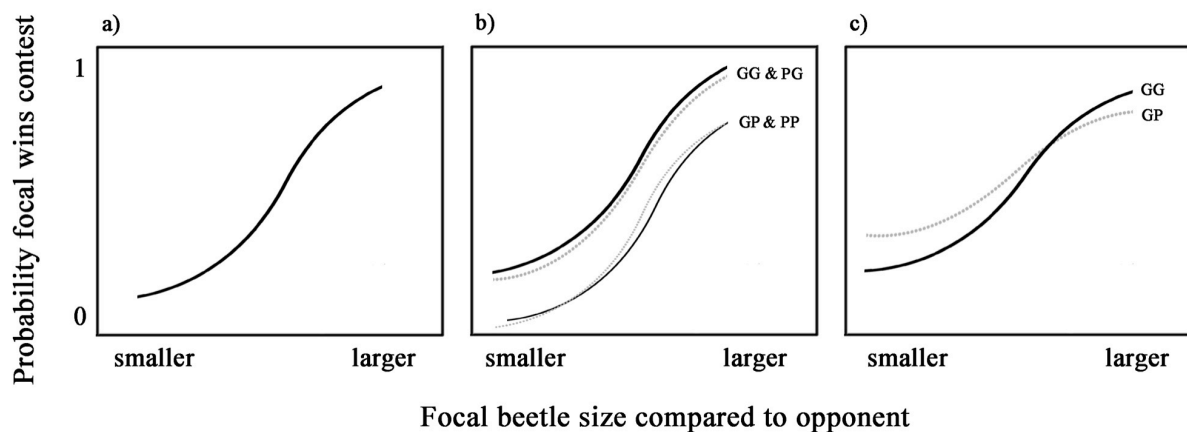
Figure 3.1: Graphical representation of key predictions. *y* axis: mean probability of victory for focal beetles, *x* axis: 'smaller' to 'larger' = increasing relative size advantage of focal beetle over opponent. Panels: a) contest success probability depends on the relative size of opponent; b) silver spoon effect of post-eclosion treatment but not larval treatment (i.e., GG and PG treatment groups have a higher probability of success than GP and PP groups for any given adult environment experienced (relative size compared to opponent)); c) environment-matching for post-eclosion delayed-feeding early-life environment (i.e., individuals in the GP treatment group fare relatively better than GG beetles in when the adult environment is poor).

Methods:

Larval nutritional environment:

Over 300 wild beetles were caught in funnel-type bottle traps baited with putrescent salmon in Devichoys wood in Cornwall, UK (SW 772 376) during the summer of 2012. Beetles were maintained and bred for four generations in accordance with Head's *et al.* (2012) methods. Three weeks after beetles eclosed, 66 virgin adult males and 66 virgin adult females were drawn from this outbred F4 stock population and randomly allocated to one another to form breeding pairs. The experimental design involved a 2 x 2 factorial manipulation of larval and post-eclosion nutritional environments as follows: For manipulation of the larval environment 33 of the 66 pairs were allocated a 'standard' sized mouse carcass of 20.76 ± 0.05g (i.e., good larval environment, 'G_') and 33 pairs a smaller carcass weighing 5.31 ± 0.05g (i.e., poor larval environment, 'P_') for use as their single available breeding resource. In total, 1511 offspring were raised from the combined 66 pairs in both larval environments. The mass of each larval nutritional environment chosen falls within the size-range of carcasses expected in the wild (e.g., juvenile and adult small mammals and songbirds) and are within the range of carcass sizes that are commonly utilized for breeding by *N. vespilloides* in the laboratory (Müller, Eggert & Furlkröger 1990; Smiseth & Moore 2002; Smiseth *et al.* 2008).

Post-eclosion nutritional environment:

As previously reported by Bartlett & Ashworth (1988) we found that broods reared on small carcasses (i.e., poor larval environment) consisted of individuals with smaller average size than did broods reared on larger carcasses (Fig. 3.2). The underlying size difference between adults reared under different larval treatments (carcass size)

was controlled to ensure that focal beetles experiencing poor larval environments (i.e., PP or PG treatment groups) did not have a higher probability of meeting an opponent larger than themselves than beetles in the good larval environment treatments groups (GP or GG). This was achieved by daily assigning all beetles eclosing from the poor larval environment to size classes (pronotum width to the nearest 0.2mm) then matching the number in each size class with individuals from good larval environments. Excess individuals, i.e., those from either larval environment with insufficient numbers of a particular size class from the alternative larval environment, were discarded (n = 791). Sufficient stock beetles were also drawn to match daily size class numbers for use as competitive trial opponents. The remaining 719 newly-eclosed beetles were allocated at random between one of two post-eclosion diets. In the first post-eclosion diet group newly-eclosed individuals were not fed for the first 6 days post-eclosion (n = 373); this delayed-feeding diet ('_P') occurred during their maturation developmental window (see Hopwood, Moore & Royle 2013). After their fast, this group was fed using our standard *ad libitum* feeding regime of two decapitated mealworm larvae, *Tenebrio molitor*, twice weekly). The second post-eclosion diet group (n = 346), in contrast, were fed mealworms immediately following eclosion and *ad libitum* thereafter ('_G'). Beetles from this second group that were both reared on larger mice and fed *ad libitum* at eclosion ('GG') effectively constituted controls that received good nutrition throughout development. In contrast 'PP' individuals experienced poor nutritional environments through development. 'PG' individuals had poor larval but good post-eclosion environments, whereas 'GP' individuals experienced good larval but poor post-eclosion environments.

Competitive trials:

A random sample of 25 animals (of both sexes) was taken from each of the four treatment groups to be used in competitive trials (i.e., total of 100 focal beetles). Stock beetles that had received standard rearing conditions throughout development (i.e., they were equivalent to the 'GG' treatment) were used as contest opponents and chosen haphazardly to eliminate bias in the direction of size differences between opposing beetles. One hundred independent intra-sexual contests were staged and filmed in the laboratory in a naturalistic setup in Nicrocosms (see appendix 1). These arenas facilitate observation of recorded video footage of individual beetle interactions over the whole prenatal period, during which time conflicts are resolved and dominance status of individuals is established. In each Nicrocosm single focal beetles, males or females, were placed simultaneously on a fresh carcass with a same-sex opponent and a stock breeding partner (i.e., a male in female-female contests and a stock female in male-male contests), during the afternoon beetle activity period between 14:00 and 17:00. In these intrasexual fights there is never a draw and the dominant individual in male-male and female-female contests was defined as the beetle that secured the carcass and succeeded in partnering with a stock individual to process the carcass (Hopwood, Moore & Royle 2013).
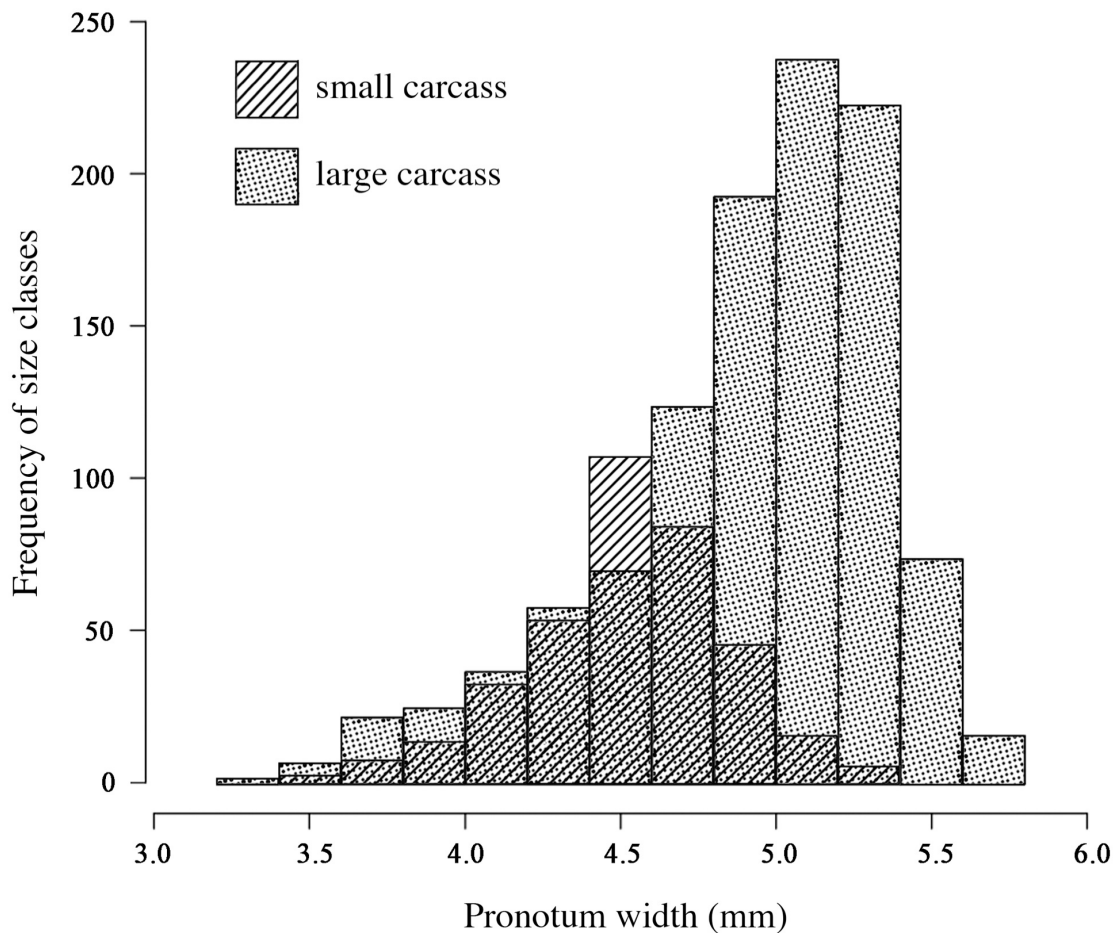
Figure 3.2: Histogram showing size distribution of all adult experimental individuals (n = 1511) from 33 families reproducing on a small carcass (poor larval environment; diagonal lines), and 33 families reproducing on a large carcass (good larval environment; dots).

Statistics:

All analyses were performed using 'R' version 2.14.1 (R Development Core Team 2011). The effect of larval environment (carcass size – large or small) on mean (within brood) adult body size was analysed using a linear model with carcass size, maternal size and paternal size as explanatory variables. Mean larval number produced per brood had a bimodal distribution and was analysed using the Wilcoxon rank sum test grouped by carcass size (large or small). The effects of the

experimental larval and post-eclosion nutritional environments experienced by individuals (2 x 2 factorial) on the probability of success in competitive trials (contest success) were analysed using a general linear model using a quasi-likelihood approach (quasi-binomial) to account for overdispersion (Crawley 2007). We included the adult (social) environment experienced (relative size of focal individual compared to its opponent) and sex as variables, testing for all 2-way interactions. The relative difference between the size of the focal beetle and its competitor was quantified using the following equation: 1 – (opponent size / focal size). This controlled for differences in the absolute size of pairs of beetles across treatments. However, our results are not dependent on this particular measure of relative size. The same terms were significant when analyses were run using absolute size difference, i.e., by subtracting the pronotal width of the focal beetle from that of its opponent. Unless stated otherwise means are presented ± 1 standard error throughout.

Results:

Effects of larval nutritional treatment on brood number and body size:

Adult body size was strongly positively related to the larval environment experienced (i.e., carcass size). Parents produced small larvae on small carcasses: brood mean offspring size (pronotum width as adult) from large carcasses = 4.95 ± 0.03mm; offspring size on small carcasses = 4.55 ± 0.04mm (LM, $F_{1,62}$ = 64.025, p < 0.0001, Fig. 3.2). Controlling for the effects of carcass size there was no statistically significant effect of maternal size ($F_{1,61}$ = 1.382, p = 0.244), paternal size ($F_{1,61}$ = 2.542, p = 0.116), or the interaction between them (maternal size × paternal size, $F_{1,59}$ = 0.037, p = 0.848) on size of offspring in adulthood.

Fewer larvae per brood were successfully reared on small carcasses (small carcasses: 12.77 ± 0.60; large carcasses: 33.85 ± 1.43; Wilcoxon rank sum test, W = 993.5, n = 64, p < 0.0001) and overall, fewer than 10% of individuals reared on small carcasses attained even the average size (i.e., 4.95mm pronotum) of those reared on large carcasses.

Effects of early-life nutritional treatment and adult social environment on success in contests:

There was a significant interaction between the quality of the adult social environment (relative size of focal beetle compared to opponent) and the post-eclosion nutritional environment experienced that determined the probability of success in contests (GLM, quasi-binomial errors, relative size difference × post-eclosion environment, $F_{1,96}$ = 4.244, p = 0.042, Fig. 3.3). Beetles that experienced a poor post-eclosion environment were more sensitive to the adult environment they experienced than individuals that had a good post-eclosion nutritional environment (the relationship had a steeper slope, Fig. 3.3). They also had a lower probability of success in contests across adult environments, unless they were considerably larger than their opponent (i.e., reduced elevation of the relationship, Fig. 3.3). All other 2-way interactions were non-significant (all p > 0.12) and larval environment *per se* (i.e., independent of its effect on adult size) was not a significant predictor of contest success (larval environment, $F_{1,95}$ = 0.003, p = 0.956). However, there was a significant main effect of the adult social environment experienced by individuals on contest outcome (relative size difference between focal beetle and its opponent: $F_{1,97}$ = 54.867, p < 0.0001), with focal beetles being more successful the larger they were

relative to their opponent as expected. There was also a non-significant trend for beetles experiencing 'poor' post-eclosion (delayed-nutrition) environments to be less likely to win contests than those reared in 'good' post-eclosion environments (post-eclosion nutritional environment, $F_{1,97}$ = 3.260, p = 0.074).
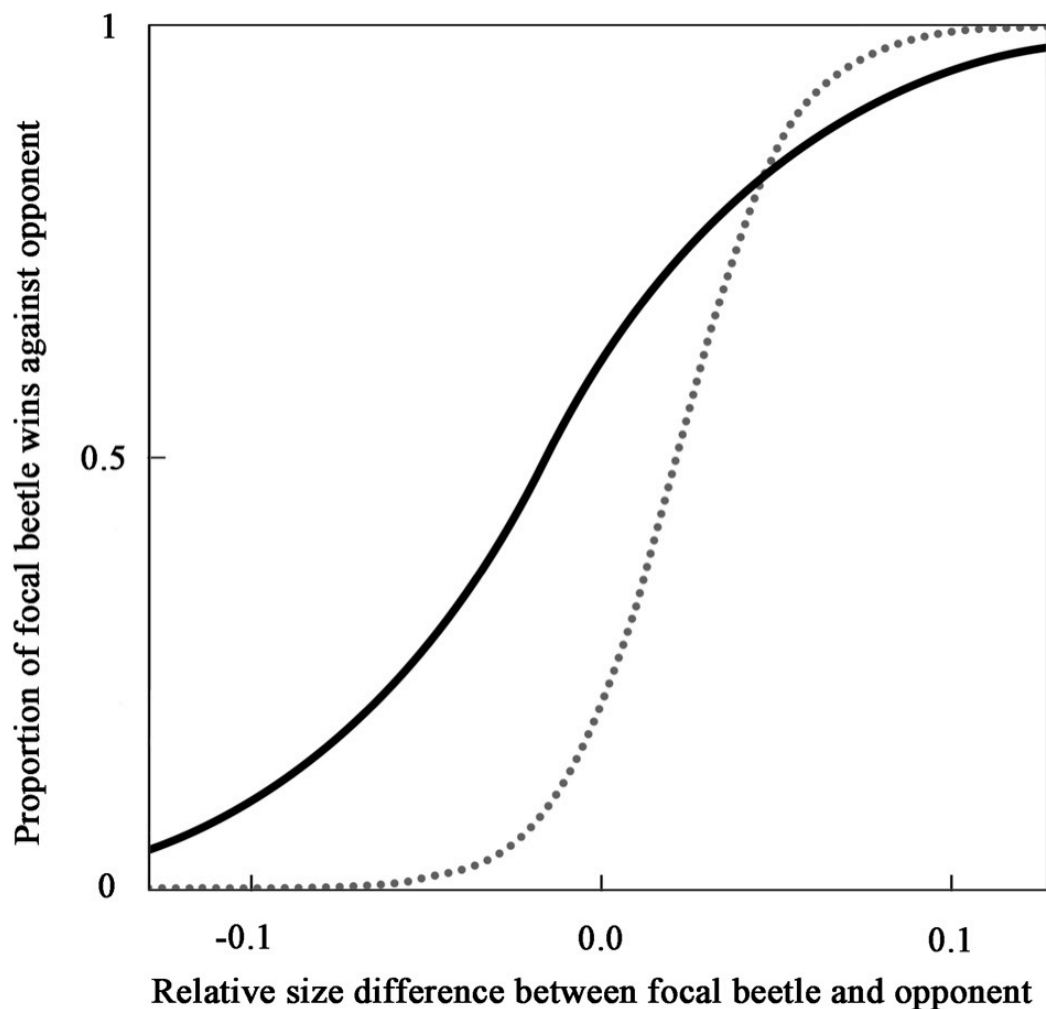
Figure 3.3: Model fit of relationship between adult environment experienced by focal individuals (relative size difference) on the *x* axis and probability of winning a contest on the *y* axis. Solid line = beetles with 'good' post-eclosion nutritional environment (i.e., fed *ad libitum* post-eclosion (GG & PG)); broken line = beetles with 'poor' post-eclosion nutritional environment (i.e., post-eclosion delayed-feeding (GP & PP)).

Discussion:

We manipulated the nutritional environment experienced by burying beetles *N. vespilloides* during two different developmental windows; first during larval development then, after pupation, during the post-eclosion maturation stage. We tested whether variation in early-life environments, at either or both of these developmental stages, predicted the probability of success during contests for breeding resources when the adult social environment also varied in quality between poor (focal individuals were small compared to their opponent) and good (focal individuals were larger than their opponent). We found a significant interaction between the post-eclosion nutritional environment experienced during development and adult social environment (size relative to opponent) predicted the probability of success in contests: the steeper slope (Fig. 3.3) of beetles that experienced poor post-eclosion nutrition indicates that they had a lower probability of winning contests across adult social environments than did beetles that experienced good post-eclosion nutrition, except when they were substantially larger than their opponents (see also Hopwood, Moore & Royle 2013). However, the effect of the larval environment (carcass size) was also important in determining the probability of winning contests for breeding resources because the size of carcass that individuals were reared on determined size at adulthood, and therefore the probability of encountering a larger opponent. As a result, although the larval environment does not affect the probability of winning a contest for a given social environment experienced in adulthood, it does affect the probability of experiencing a poor quality adult environment (i.e., encountering an opponent larger than itself), which in turn affects contest outcome. There were therefore both direct (post-eclosion environment affected competitive ability independently of body size) and indirect

(larval environment affected contest success via the likelihood of meeting a larger rival) effects of variation in nutrition during development on the probability of winning contests in adulthood.

In burying beetles reproduction depends upon finding and securing access to a carcass of a small vertebrate. As the availability of carcasses suitable for breeding is likely to be limited and their distribution ephemeral, direct contests over breeding resources are common (Müller, Eggert & Dressel 1990). Body size and condition of the individual are known to be important determinants of success in contests (Bartlett & Ashworth 1988; Otronen 1988; Hopwood, Moore & Royle 2013), so the quality of the adult (social) environment that individuals experience can be defined in terms of their size compared to that of their opponents, from good (larger than opponent) to poor (smaller than opponent). Studies of developmental nutritional variation at different life stages often look for interactions between juvenile and adult experience, but rarely, if ever, consider early-life effects on adult phenotypes expressed in the context of social competition (Monaghan 2008). By independently manipulating the quality of the nutritional environment at two different stages of development and varying the quality of the adult social environment experienced we could test whether early-life environments predict success in later competitive social environments. The question then is: Do the data support an environmental-matching hypothesis or fit a silver spoon scenario?

Environmental-matching or silver spoon?
For environmental-matching to be supported we would expect individuals that had a poor nutritional environment during the larval stage (individuals reared on small

carcasses) and/or post-eclosion stage to be better relative competitors in poor adult environments (i.e., when smaller than their opponent, Fig. 3.1c). In addition, evidence for an adaptive strategy should fulfil the requirement that early environmental conditions during development reliably predict later environmental conditions. Pupating at a relatively small size might provide such a cue: there will be a higher likelihood of encountering a rival of greater size than itself. This would represent a potential serious disadvantage in securing or defending a breeding resource. However, we found no evidence that beetles that experienced poor early-life environments (i.e., beetles from the PG, GP or PP groups), had a greater relative probability of contest success when the adult environment was poor compared to beetles that had good nutritional environments throughout development (GG group) (see also Safryn & Scott 2000). In our experiment the probability of contest success for adults depended not only on the quality of the adult social environment (relative size of focal compared to opponent) but also on an interaction with the post-eclosion environment. However, the direction of the effect of the interaction was opposite to that predicted by environmental-matching. Individuals reared under poorer post-eclosion environments (_P) did even worse than those with good post-eclosion environments (_G) when adult environments were poor (i.e., from an adaptive perspective, these individuals would be environmentally mismatched, Fig. 3.3). There was therefore no evidence in support of environmental-matching.

Did poor early nutrition disrupt optimal development leading to a silver spoon effect? Beetles experiencing good post-eclosion environments (access to *ad libitum* food: GG and PG treatment groups) had better relative performance during contests for a given quality of adult environment than did those that experienced post-eclosion

delayed-feeding (PP and GP treatment groups; Fig. 3.1b & Fig. 3.3), except when adult environments were very good (i.e., when much larger than their opponent). This indicates a context-dependent, direct silver spoon effect.

In contrast to the effects of variation in nutrition experienced post-eclosion, variation in the nutritional environment experienced during the larval stage did not affect the probability of success during contests for a given quality of adult environment (i.e., controlling for body size differences). However, because body size is closely related to the quality of the larval nutritional environment there is likely to be a close association between competitive ability in adulthood and the size of the carcass on which individuals develop. Large size relative to opponents was a primary determinant of success in adult contests for breeding resources, as has also been documented in other studies of this species (Bartlett & Ashworth 1988; Otronen 1988; Hopwood, Moore & Royle 2013), and widely reported across other taxa (Schuett 1997; McElligott *et al.* 2001; Briffa 2008; Reaney, Drayton & Jennions 2011).

Larvae developing on small carcasses were themselves small as adults (Fig. 3.2) so in nature these groups of beetles are likely to occupy different parameter space with respect to the likely adult social environments they experience. In the wild the importance of being small in contests for breeding resources may depend on the size distribution of individuals in the population. Assuming a normal distribution of the availability of carcass sizes in the wild, because broods reared on small mice contain smaller individuals and fewer individuals, the size distribution of adult beetles is likely to be skewed towards relatively large beetles. Consequently larvae developing on

small carcasses suffer a disproportionally high probability of encountering rivals of greater size than themselves. As a result, in addition to the direct silver spoon effects of variation in quality of the post-eclosion environment on contest success there are also indirect silver spoon effects on contest outcomes of variation in the nutritional environment experienced during the larval stage: larvae reared on small carcasses are not competitively inferior *per se* (i.e., for a given adult social environment), but are more likely to experience poor adult social environments (encounter an opponent larger than themselves) because they are small. However, both of these effects are context-dependent, with the direct effect only occurring when the adult environment is poor and the indirect effect only occurring when the adult environment is good, so neither can be considered simple, clear-cut silver spoon effects (Blount *et al.* 2006; Taborsky 2006; Van De Pol *et al.* 2006; Dmitriew & Rowe 2011; Krause & Naguib 2014).

The advantage of large size may only be realised in the context of competition at a carcass. In *N. vespilloides*, larger males mating with polyandrous females have no advantage over smaller males when mating away from a carcass (i.e., sperm competition without any immediate male-male competition) but smaller males suffer a disadvantage when both males mated on a carcass (House, Hunt & Moore 2007). When the population is dense and there are many competitors for carcasses, larger individuals may have an advantage. Moreover, when suitable carcasses are more abundant, large individuals may be at an advantage by having the potential to produce more broods with greater numbers than can smaller beetles (Bartlett 1988). However, small beetles could mitigate their disadvantage in contest ability by avoiding contests, for example, by being the first beetle to locate a carcass and/or

preferentially attracting females. If they are subsequently usurped from ownership of a carcass by a larger individual they may still resort to alternative reproductive strategies such as brood parasitism or satellite behaviour and/or return to the mating-pool early. There may also be direct general benefits of a smaller body size such as lower costs of flight and lower overall maintenance costs (Blanckenhorn 2000) that might enable wider or more prolonged searches for resources. Smaller individuals may not be at a reproductive disadvantage when carcasses are small or poor quality and intriguingly, there is evidence that they can produce broods with offspring substantially larger than themselves (Bartlett & Ashworth 1988).

Fitness implications of offspring size and nutritional variation in burying beetles are unknown. In burying beetles body size is mediated by the caring behaviour of parents as broods are tailored to match the size of the breeding carcass, i.e., fewer larger larvae, or more but smaller larvae may be reared (Bartlett 1987; Eggert & Müller 1997). This leads to the possibility that producing a brood of smaller (or larger) than average offspring might be a parental adaptive response to the likelihood of future hard times for their offspring. Van De Pol *et al*. (2006) used a long-term dataset to study transgenerational effects of natal origin (high quality or low quality habitat patches) on fitness in wild oystercatchers, *Haematopus ostralegus*. By measuring fitness both as individual components (e.g., survival to adulthood and recruitment) and also combined components (e.g., relative output per fledgling from each habitat through subsequent breeding years) it emerged that long-term effects of the early environmental conditions were as important as short-term effects in this species. One of the drivers of the long-term effects was the increased likelihood that offspring reared on high-quality patches would themselves secure

good breeding habitat as adults. However, burying beetles don't face the same choice between low and high quality breeding patches; all individuals must vary their tactics depending on whether a carcass is poor or good, and contested or uncontested. Nevertheless, their reproductive success depends on interactions between the effect of their early environment and the social environment they experience in adulthood. Unpredictability in both the nutritional and social environment they face may help to explain the extraordinary variation in size and mating tactics in *Nicrophorus vespilloides*.

Conclusions:

Our results indicate that effects of variation in developmental conditions on success in adulthood may be complex and dependent on the ecological context in which they are expressed. There was no evidence for environmental-matching or simple silver spoon effects. Instead results showed that benefits of good nutrition during development depended on the adult social environment individuals experienced. Adult beetle size is permanently influenced by the size of the carcass on which individuals develop; beetles reared on small carcasses are smaller than beetles reared on larger carcasses. Body size is the most important predictor of contest success for breeding resources, so a large carcass represents an 'indirect' silver spoon for the brood that it supports. However, benefits of a good larval environment are dependent on the social environment that adults experience because beetles reared on smaller carcasses fared no worse against opponents than did those reared on larger carcases, for a given size differential between individuals. When beetles had good nutrition post-eclosion compared to a delay in feeding they benefitted from a 'direct' silver spoon effect on contest success. However their

advantage was only evident when the adult environment was poor (i.e., they met an opponent larger than themselves). Being small is also not necessarily disadvantageous for burying beetles. Size-dependent success may be influenced by breeding resource unpredictability, population density, variation in the proportion of contested carcasses, sexual selection and carcass discovery all of which need to be investigated in the wild. This may be key to understanding the evolution and maintenance of the plasticity in body size and reproductive strategy found in *N. vespilloides*.

References:

Barrett, E.L.B., Hunt, J., Moore, A.J. & Moore, P.J. (2009) Separate and combined effects of nutrition during juvenile and sexual development on female life-history trajectories: the thrifty phenotype in a cockroach. *Proceedings of the Royal Society B,* **276,** 3257-3264.

Barrett, E.L.B., Moore, A.J. & Moore, P.J. (2009) Diet and social conditions during sexual maturation have unpredictable influences on female life history trade-offs. *Journal of Evolutionary Biology,* **22,** 571-581.

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

Bartlett, J. (1988) Male mating success and paternal care in *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Behavioral Ecology and Sociobiology,* **23,** 297-303.

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

Beeler, A.E., Rauter, C.M. & Moore, A.J. (1999) Pheromonally mediated mate attraction by males of the burying beetle *Nicrophorus orbicollis*: alternative calling tactics conditional on both intrinsic and extrinsic factors. *Behavioral Ecology,* **10,** 578-584.

Benton, T.G., Plaistow, S.J., Beckerman, A.P., Lapsley, C.T. & Littlejohns, S. (2005) Changes in maternal investment in eggs can affect population dynamics. *Proceedings of the Royal Society B: Biological Sciences,* **272,** 1351-1356.

Birkhead, T.R., Fletcher, F. & Pellatt, E.J. (1999) Nestling diet, secondary sexual traits and fitness in the zebra finch. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* **266,** 385-390.

Blanckenhorn, W.U. (2000) The evolution of body size: what keeps organisms small? *Quarterly Review of Biology,* **75,** 385-407.

Blount, J.D., Metcalfe, N.B., Arnold, K.E., Surai, P.F. & Monaghan, P. (2006) Effects of neonatal nutrition on adult reproduction in a passerine bird. *Ibis,* **148,** 509-514.

Briffa, M. (2008) Decisions during fights in the house cricket, *Acheta domesticus*: mutual or self assessment of energy, weapons and size? *Animal Behaviour,* **75,** 1053-1062.

Brisson, J.A. & Stern, D.L. (2006) The pea aphid, *Acyrthosiphon pisum*: an emerging genomic model system for ecological, developmental and evolutionary studies. *Bioessays,* **28,** 747-755.

Butler, M.W. & McGraw, K.J. (2012) Differential effects of early- and late-life access to carotenoids on adult immune function and ornamentation in mallard ducks (*Anas platyrhynchos*). *PloS one,* **7,** e38043.

Crawley, M.J. (2007) *The R book*. Wiley and Sons, Chichester.

Descamps, S., Boutin, S., Berteaux, D., McAdam, A.G. & Gaillard, J.M. (2008) Cohort effects in red squirrels: the influence of density, food abundance and temperature on future survival and reproductive success. *Journal of Animal Ecology,* **77,** 305-314.

Dmitriew, C. & Rowe, L. (2011) The effects of larval nutrition on reproductive performance in a food-limited adult environment. *PloS one,* **6,** e17399.

Eggert, A.K. & Müller, J.K. (1997) Biparental care and social evolution in burying beetles: lessons from the larder. *The evolution of social behavior in insects and arachnids* (eds J.C. Choe & B.J. Crespi), pp. 216-236. Princeton University Press, Princeton.

Emlen, D.J. (2000) Integrating development with evolution: a case study with beetle horns. *Bioscience,* **50,** 403-418.

Gilbert, S.F. (2005) Mechanisms for the environmental regulation of gene expression: ecological aspects of animal development. *Journal of Biosciences,* **30,** 65-74.

Gluckman, P.D., Hanson, M.A. & Spencer, H.G. (2005) Predictive adaptive responses and human evolution. *Trends in Ecology & Evolution,* **20,** 527-533.

Grafen, A. (1988) On the uses of data on lifetime reproductive success. *Reproductive success* (ed. T.H. Clutton-Brock), pp. 454-471. University of Chicago Press, Chicago.

Head, M.L., Berry, L.K., Royle, N.J. & Moore, A.J. (2012) Paternal Care: Direct and indirect genetic effects of fathers on offspring performance. *Evolution,* **66,** 3570-3581.

Hopwood, P.E., Moore, A.J. & Royle, N.J. (2013) Nutrition during sexual maturation affects competitive ability but not reproductive productivity in burying beetles. *Functional Ecology,* **27,** 1350-1357.

House, C.M., Hunt, J. & Moore, A.J. (2007) Sperm competition, alternative mating tactics and context-dependent fertilization success in the burying beetle, *Nicrophorus vespilloides*. *Proceedings of the Royal Society B,* **274,** 1309-1315.

Krause, E.T. & Naguib, M. (2014) Effects of parental and own early developmental conditions on the phenotype in zebra finches (*Taeniopygia guttata*). *Evolutionary Ecology,* **28,** 263-275.

Lee, V.E., Head, M.L., Carter, M.J. & Royle, N.J. (2013) Effects of age and experience on contest behaviour in the burying beetle, *Nicrophorus vespilloides*. *Behavioral Ecology,* **25,** 172-179.

McElligott, A.G., Gammell, M.P., Harty, H.C., Paini, D.R., Murphy, D.T., Walsh, J.T. & Hayden, T.J. (2001) Sexual size dimorphism in fallow deer (*Dama dama*): do larger, heavier males gain greater mating success? *Behavioral Ecology and Sociobiology,* **49,** 266-272.

Monaghan, P. (2008) Early growth conditions, phenotypic development and environmental change. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **363,** 1635-1645.

Moore, A.J., Brodie III, E.D. & Wolf, J.B. (1997) Interacting phenotypes and the evolutionary process: I. direct and indirect genetic effects of social interactions. *Evolution,* **51,** 1352-1362.

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

Müller, J.K., Eggert, A.K. & Dressel, J. (1990) Intraspecific brood parasitism in the burying beetle, *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Animal Behaviour,* **40,** 491-499.

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

Nijhout, H.F. (2003) Development and evolution of adaptive polyphenisms. *Evolution & Development,* **5,** 9-18.

Nussey, D.H., Kruuk, L.E.B., Morris, A. & Clutton-Brock, T.H. (2007) Environmental conditions in early life influence ageing rates in a wild population of red deer. *Current Biology,* **17,** R1000-R1001.

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

R Development Core Team (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org.

Reaney, L.T., Drayton, J.M. & Jennions, M.D. (2011) The role of body size and fighting experience in predicting contest behaviour in the black field cricket, Teleogryllus commodus. *Behavioral Ecology and Sociobiology,* **65,** 217-225.

Royle, N.J., Lindström, J. & Metcalfe, N.B. (2005) A poor start in life negatively affects dominance status in adulthood independent of body size in green swordtails *Xiphophorus helleri*. *Proceedings of the Royal Society B: Biological Sciences,* **272,** 1917-1922.

Saastamoinen, M., Van der Sterren, D., Vastenhout, N., Zwaan, B.J. & Brakefield, P.M. (2010) Predictive adaptive responses: condition-dependent impact of adult nutrition and flight in the tropical butterfly *Bicyclus anynana*. *The American Naturalist,* **176,** 686-698.

Saeki, Y. & Crowley, P.H. (2013) The size-number trade-off in clonal broods of a parasitic wasp: responses to the amount and timing of resource availability. *Functional Ecology,* **27,** 155-164.

Safryn, S.A. & Scott, M.P. (2000) Sizing up the competition: Do burying beetles weigh or measure their opponents? *Journal of Insect Behavior,* **13,** 291-297.

Schuett, G.W. (1997) Body size and agonistic experience affect dominance and mating success in male copperheads. *Animal Behaviour,* **54,** 213-224.

Segers, F.H.I.D. & Taborsky, B. (2011) Egg size and food abundance interactively affect juvenile growth and behaviour. *Functional Ecology,* **25,** 166-176.

Smiseth, P.T., Hwang, W., Steiger, S. & Müller, J.K. (2008) Adaptive consequences and heritable basis of asynchronous hatching in *Nicrophorus vespilloides*. *Oikos,* **117,** 899-907.

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

Spear, L. & Nur, N. (1994) Brood size, hatching order and hatching date: effects on four life-history stages from hatching to recruitment in western gulls. *Journal of Animal Ecology,* **63,** 283-298.

Taborsky, B. (2006) The influence of juvenile and adult environments on life-history trajectories. *Proceedings of the Royal Society B,* **273,** 741-750.

Uller, T. (2012) Parental effects in development and evolution. *The evolution of parental care* (eds N.J. Royle, P.T. Smiseth & M. Kölliker), pp. 247-266. Oxford University Press, Oxford.

Van De Pol, M., Bruinzeel, L.W., Heg, D., Van Der Jeugd, H.P. & Verhulst, S. (2006) A silver spoon for a golden future: long-term effects of natal origin on fitness prospects of oystercatchers (*Haematopus ostralegus*). *Journal of Animal Ecology,* **75,** 616-626.

Waterland, R.A. & Jirtle, R.L. (2004) Early nutrition, epigenetic changes at transposons and imprinted genes, and enhanced susceptibility to adult chronic diseases. *Nutrition,* **20,** 63-68.

Chapter 4:

Male burying beetles extend parental care duration when threats to paternity assurance increase.

Summary:

Male parents tend to desert before females in many species with biparental care. The dominant theory to explain male desertion is that it occurs because males are unsure about whether they are the genetic parent of the offspring for which they caring: the less sure they are, the more likely they are to desert the brood in favour of pursing other mating opportunities. Recently it has been suggested that male care may evolve because staying to care actively improves paternity via reducing cuckoldry or increasing success in sperm competition, rather than primarily because it increases offspring fitness. If males benefit from increased paternity by staying to care one prediction is that threats to confidence in paternity will be associated with increased paternal care. We manipulated the perceived threat to paternity directly with different sex-ratios of competitors in biparental reproductive bouts of burying beetles, *Nicrophorus vespilloides*. Males deserted later when there were male challengers for paternity and/or opportunities to mate with additional females. Caregiving males increased mating frequency (a paternity assurance behaviour) in response to male competition. We found no evidence that offspring benefitted from extended male care and the increased mating frequency of males facing competitors delayed the safe interment of the breeding resource. Our findings support the increased paternity hypothesis. In burying beetles, extended duration of care by males may be a tactic to maximise paternity (in the current brood and in subsequent

broods in which females use stored sperm) even when this fails to maximise offspring fitness and creates a conflict of interest with their mate.

Introduction:

In most species with biparental care females spend more time caring than males and the reason why male parents desert before females and why they vary in the length of time they stay with their caring partner is often unclear (Kokko & Jennions 2012). Regardless of intersexual differences in prenatal reproductive costs (such as egg versus sperm production) offspring fitness benefits associated with increased postnatal parental effort should be shared by both parents. However, while both parents are likely to share a genetic interest in the brood, neither are likely to be as closely related to each other as they are to their offspring (Trivers 1972; Arnqvist & Rowe 2005).

Conflicts of interest between parents occur because parental care is costly: it takes time that could be used in trying to find more mating opportunities, and energy invested in current offspring may trade-off with future reproductive productivity (Clutton-Brock 1991; Royle, Smiseth & Kölliker 2012). Because of these costs each individual parent stands to gain in the future if they can minimize their own current effort at the expense of their partner (Trivers 1972; Parker, Royle & Hartley 2002). There is empirical evidence for individual parents adjusting their own level of effort according to the level provided by their partner, but often the response only partially

compensates (Harrison *et al.* 2009). Royle *et al.* (2002) found that compared to caring uniparentally, zebra finches, *Taeniopygia guttata*, reduced individual parental investment when providing care together with their partner (when the number of offspring per parent was controlled). This led to a reduction in care per individual offspring under biparental care, and offspring raised by single females were more sexually attractive as adults compared to those raised by both parents (Royle, Hartley & Parker 2002).

This paradigm has been used to explain variation in the level of male care: when care is costly and is about investment in offspring, males that care for offspring unrelated to themselves will be selected against (Whittingham, Taylor & Robertson 1992; Houston 1995; Kokko & Jennions 2008; Alonzo & Klug 2012). Hence, within species, cues indicating declining paternity assurance are expected to be associated with reduced paternal care and empirical support comes from studies showing males decreasing parental effort when they obtain fewer matings (e.g., Hartley *et al.* 1995) or when risks of cuckoldry increase (e.g., Hunt & Simmons 2002). However, there are exceptions where males make large investments in care despite low paternity (Neff 2003; Griffin, Alonzo & Cornwallis 2013). For example, terminal investment can result in older males increasing effort in a current brood in response to decreasing likelihood of any further reproductive opportunity (Scott 1998b; Benowitz *et al.* 2013).

An alternative hypothesis to the general prediction that males will stay and care for offspring likely to be their own is that male care may evolve not because it allows males to invest in their offspring, but rather because it increases their paternity assurance (Kvarnemo 2006; Kahn, Schwanz & Kokko 2013). Thus, males that stay

and care benefit from opportunities actively to increase paternity assurance. The increased paternity hypothesis casts male parental care in a different light: not only does male care no longer need to be beneficial to offspring, it may also create additional conflicts of interest between mates. Reproductive competitors could simultaneously represent a threat to a male parent through loss of paternity but be neutral, or even present an opportunity, for his female partner. Therefore, male parental investment decisions might vary, independently of shared fitness interests (i.e., offspring fitness *per se*) reflecting instead the level of competition in the social environment in which parental traits are expressed. A challenge to offspring paternity from potential cuckolds provides motivation for a male to stay and ensure as many offspring as possible are his own; male care should be extended when this increases paternity assurance in the current or future broods (Kvarnemo 2006; Kahn, Schwanz & Kokko 2013).

Burying beetles provide a model system with which these ideas can be tested. Male and female parents appear to cooperate and share duties for the benefit of their combined offspring. Both sexes provide complex prenatal and postnatal care, either alone or together and, for uniparental care, male and female parental behaviour has been shown to increase offspring fitness (Scott 1989; Eggert, Reinking & Müller 1998; Smiseth, Darwell & Moore 2003; Smiseth, Lennox & Moore 2007). In the wild however, burying beetles often face stiff competition for small vertebrate carcasses that are both a necessary and sufficient resource for a single breeding event (Pukowski 1933). Carcasses are valuable and beetles losing contests for dominant reproductive status often remain and adopt satellite (male) and brood parasitic (female) roles. These competitors lead to uncertainty for both parents over their

genetic parentage of a brood—and critically, the sex ratio and extent of this competition varies among reproductive events (Müller, Eggert & Dressel 1990; Müller *et al.* 2007). This means that until all beetle eggs around a carcass are laid, the threat that competitors pose to each parent depends on their sex. Eggs of a brood parasitic female (or females) dilute the dominant female's parentage but the dominant male may have sired the resulting offspring. In contrast satellite males represent a threat to the paternity of the dominant male by sneaking matings with the females present (Scott 1998a; Müller *et al.* 2007). These imbalances are reflected in the exclusively intrasexual fights that establish dominance at a carcass (Otronen 1988; Lee *et al.* 2013).

Studies on burying beetles have acknowledged the importance of intrasexual competition on parentage during a breeding bout (e.g., Müller *et al.* 2007; Müller & Eggert 1989) but have not tested the effect that variation in this competitive social environment has on parental behaviour and the effect on reproductive output. We manipulated the sex ratio of reproductive competitors in the burying beetle *N. vespilloides* to test a critical prediction of the increased paternity hypothesis: that male care should be extended only when this is likely to increase paternity assurance (Kvarnemo 2006). If male decisions to stay and care are based on returns via offspring fitness, males should stay longer when in a monogamous pair than when there is competition and greater duration of care should be positively correlated with fitness components of offspring. In contrast, if as predicted by Kvarnemo (2006), male care decisions are based on increasing paternity (i.e., securing a greater proportion of parentage through guarding and repeated mating),

the opposite pattern should be seen: males will stay longer when there is intrasexual

competition at the carcass with no, or negative, effects on offspring fitness.

Methods:

Over 200 wild beetles were attracted to baited (small pieces of putrescent salmon)

funnel-type bottle-traps hung in trees in a Cornish woodland, UK (SW 772 376)

during the autumn of 2011. Laboratory experimental stock was generated from the

outbred F4 offspring of these wild beetles. Beetles were housed individually in clear

plastic boxes, maintained at a temperature of 16 – 20°C with an 16:8hr light:dark

cycle and fed decapitated mealworms *ad libitum* from eclosion until introduced to

their experimental environments as described by Head *et al.* (2012).

246 laboratory stock beetles were weighed, measured and randomly assigned to

one of four treatments. Each beetle within each treatment replicate was marked to

enable identification of individuals. Marking was achieved by lightly scratching a

small area (~1mm Ø) on the dorsal surface of elytra with a hobby-drill fine sanding-

bit, in one of the four distinctive orange patches (i.e., either rear right; rear left; front

right or front left), and then applying a dot of black Indian ink. The orange quarter to

be marked was randomized for each of the four (or two) individuals within each

treatment group and replicate.

The whole duration of each breeding bout was filmed in the laboratory (from the

introduction of beetles to a carcass until larvae dispersed) using arenas designed for

this purpose. Each arena consisted of a 400mm length of black PVC-U 110mm Ø

pipe placed upright in a 30L plasterer's bucket containing approximately 25mm of

moist compost. Three 40mm Ø exit holes were drilled in the inner pipe 5mm above the compost surface level (these inner pipes we refer to as 'Nicrocosms' while 'arena' refers to Nicrocosm and bucket combined). A closed-circuit, infra-red surveillance camera (N08CX night vision CCTV camera) was positioned inside the lumen of each Nicrocosm using motion detecting software (AVerMedia NV6240 Express, DVR version 7.7.0.0007; www.avermedia-dvrs.com) to capture beetle activity around a mouse carcass that was positioned inside (see appendix 2). Experimental female beetles were mated with non-experimental stock males 24 hours before being used, as almost all (93%) sexually mature females caught in the wild have been found to have pre-mated (Müller & Eggert 1989). Experimental beetles were assigned at random to treatment groups when they were between 14 and 21 days old (post eclosion) but kept separate from one another until introduced to the arena. Mouse size was standardized (21.16 ± 0.07g) across treatments to enable comparisons of reproductive output.

Treatment groups were:

Female bias: Three females and one male (replicates: $n = 17$) had access to a single mouse carcass. The virgin male was placed in the arena with a mouse carcass, and three randomly chosen pre-mated females, during the activity period in the afternoon (when wild beetles fly in search of carcasses).

Male bias: One pre-mated female was introduced to three virgin males and a mouse carcass during the afternoon activity period ($n = 18$).

No sex-bias: Two pre-mated females and two virgin males were placed in an arena containing a mouse carcass ($n = 17$).

No current extra-pair competition): A virgin male and a single pre-mated female were placed in an arena with a mouse carcass (*n* = 19).

Beetle activity on and around the carcass (i.e., inside the Nicrocosm) was recorded on video from the time experimental beetles were introduced to carcasses until larval dispersal. Duration of care was measured by recording the time and identity of parental desertion (desertion defined as an unbroken six hour absence from the carcass with no parental behaviour witnessed). Desertion was quantified for analysis as the proportion of postnatal care that a beetle stayed between the first larvae seen and larval dispersal from the carcass. Number of matings was used as a measure of paternity assurance behaviour. Two indicators of prenatal parental performance were used: firstly, latency until commencing carcass burial was measured from the time first contact was made with the carcass for individuals (defined as physical contact with carcass combined with exploratory behaviour, i.e., stopping and waving antennae or burrowing under carcass) until commencement of interment (physical contact with carcass resulting in its first purposeful displacement - usually movement of the carcass head). Secondly, the duration of burial was measured from commencement of interment to the time that only the distal half of the mouse tail remained above ground. Postnatal parental performance was measured both in terms of brood mass at larval dispersal and as larval developmental duration: the time of first larva seen walking above ground towards the carcass until at least two fully-developed larvae left the carcass - usually swiftly followed by the brood *en masse*). Dominant beetles were designated as the male and female that repelled initial same-sex incursions on to the carcass; that subsequently engaged in prenatal preparation of the carcass; that predominantly remained inside the Nicrocosm; and

that became the postnatal parental care providers (except in the case of two dominant males which deserted prior to larval hatching but fulfilled the other criteria). In treatments with no same-sex competition the uncontested beetle is always designated a dominant carer.

Statistics

Analyses were performed using 'R' version 2.14.1 (R Development Core Team 2011). To test whether male duration of care was related to likelihood of paternity assurance, male duration of care (the proportion of total postnatal larval developmental time spent performing parental duties) was analysed using a generalized linear model with a quasi-binomial error structure with social treatment and dominant male and female (i.e., carers') body size included as independent variables. To test whether the sex likely to desert first was predicted by treatment cues to likely parentage proportion, a count of first desertion by sex-of-parent among treatments was analysed with a Fisher's exact test. Males variation in paternity assurance behaviour was analysed using a linear model using mating frequency (log-transformed to normalise its distribution) and. Independent variables were treatment, male carer size and female carer size and the effect of variation in mating frequency on prenatal parental performance was examined by analysing carcass burial duration. Parental performance in terms of total brood mass was analysed using a linear model with treatment, proportion of time male stayed postnatally and the difference between male and female caregivers size as independent variables. Brood size (larval number) was analysed using a GLM with quasi-Poisson error structure with treatment, and the difference between male and female caregivers size as independent variables. Minimal adequate models and significance were

determined through stepwise model simplification (Crawley 2007). Post-hoc multiple comparisons where necessary were obtained using Tukey's honestly significant differences.

Results: Dominant male parents adjusted their desertion decisions according to the social competitive environment experienced at a carcass: they deserted significantly earlier from monogamous pairs compared to carcasses where there was any intrasexual competition (GLM with quasi-binomial error structure: treatment, $F_{3,62} = 4.423$, $p = 0.007$, Fig. 4.1). The size of the dominant male did not affect the duration of his postnatal care ($F_{1,61} = 0.765$, $p = 0.385$) but longer male care was associated with having a larger female partner ($F_{1,62} = 5.997$, $p = 0.017$). The sex of the dominant carer that deserted first was not influenced by the social competitive environment (Fisher exact test: $n = 69$, $p = 0.319$); all but three dominant females remained with their broods until the broods dispersed regardless of the nature or sex ratio of competition.
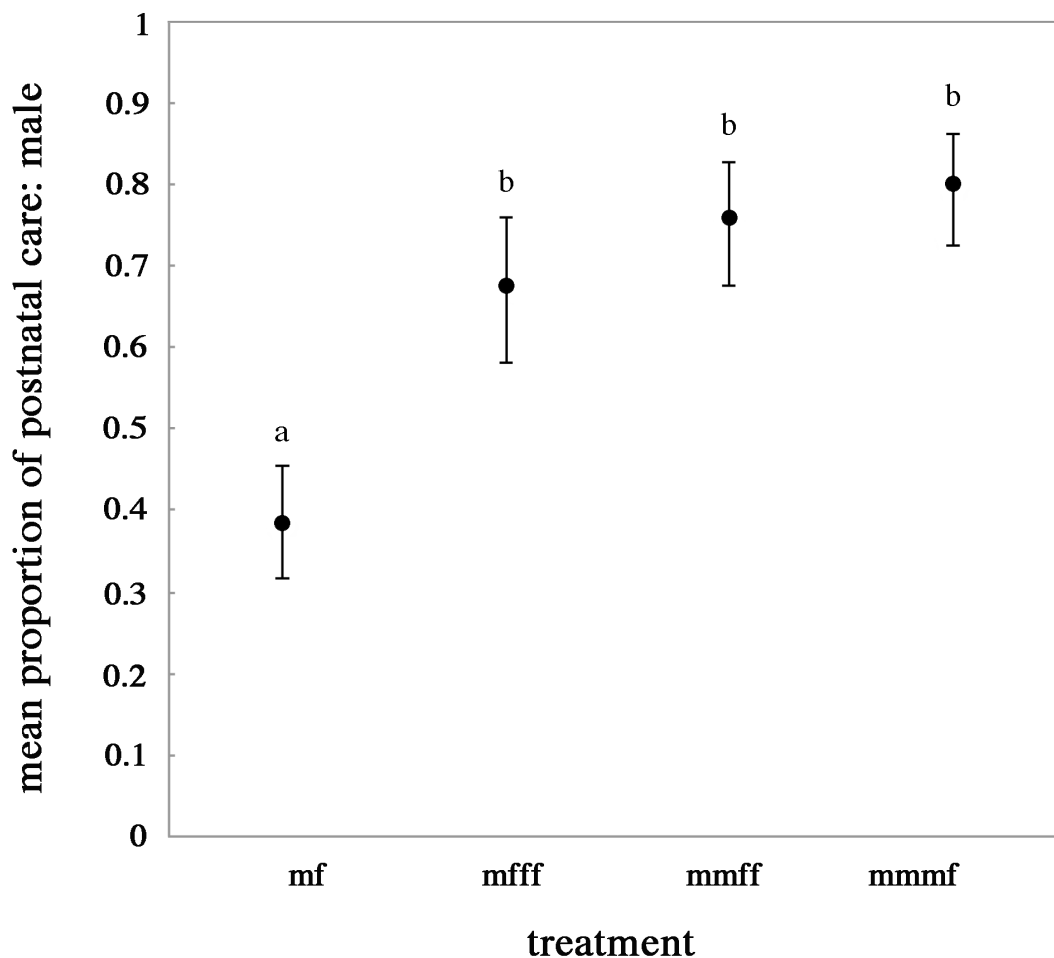
Figure 4.1: Mean proportion of the total postnatal larval developmental period (from first larval arrival at carcass to larval dispersal from carcass) that the dominant male remained in attendance performing parental activities. *x*-axis labels indicate the constituents of four different social competitive treatments (i.e., 'mf' = one male and one female; 'mfff' = one male and three females; 'mmff' = two males and two females; 'mmmf' = three males and one female). Lower case letters (a & b) indicate significant differences among treatment groups.

Social environment effects on reproductive productivity

Variation in the social competitive environment affected reproductive productivity: broods were heavier at dispersal in the treatment without adult competition than when extra males competed for matings with a single female (LM: treatment, $F_{3,64}$ = 3.203, p = 0.029, Fig. 4.2). In addition to treatment, the relative size difference between male and female dominant parents had an effect on brood weight at

dispersal: the larger the female partner relative to her male caring partner, the larger was the mean weight of the whole brood (parental size relative difference, $F_{1,64}$ = 6.721, p = 0.012). The duration of male postnatal care had no significant effect on brood mass ($F_{1,65}$ = 0.050, p = 0.823).
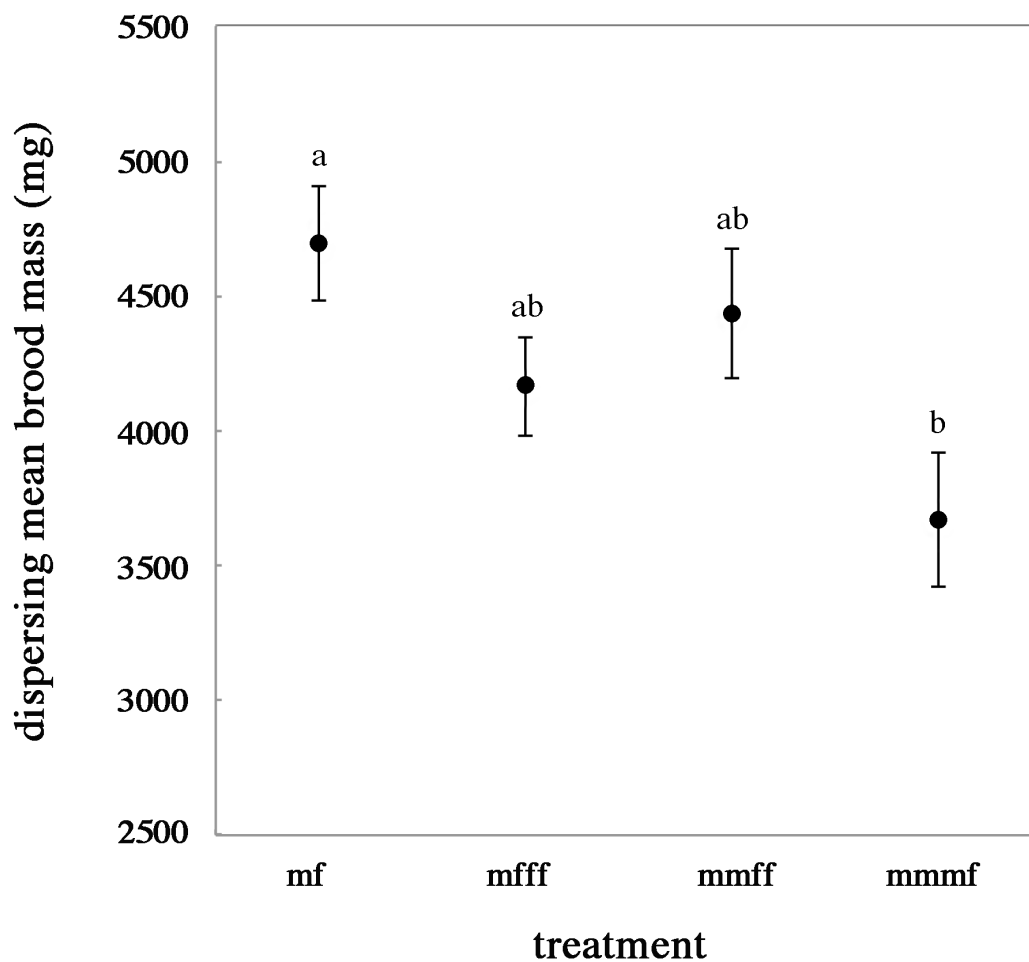
Figure 4.2: Effect of treatment on mean brood mass at dispersal. Lower case letters (a & b) indicate significant differences among treatment groups.

Social environment effects on mating frequency:

The social environment treatment affected the number of times that the focal male mated with the focal female: dominant males with male competitors present mated

more frequently with the dominant female than did males in monogamous pairs (LM, treatment, $F_{3,62} = 4.667$, $p = 0.005$, Fig. 4.3). In the two treatments with male competitors mating frequency of the dominant male was significantly greater than that of subordinates (Wilcoxon signed rank test, dominant status, $V = 525$, $n = 37$, $p < 0.0001$, Fig. 4.3).
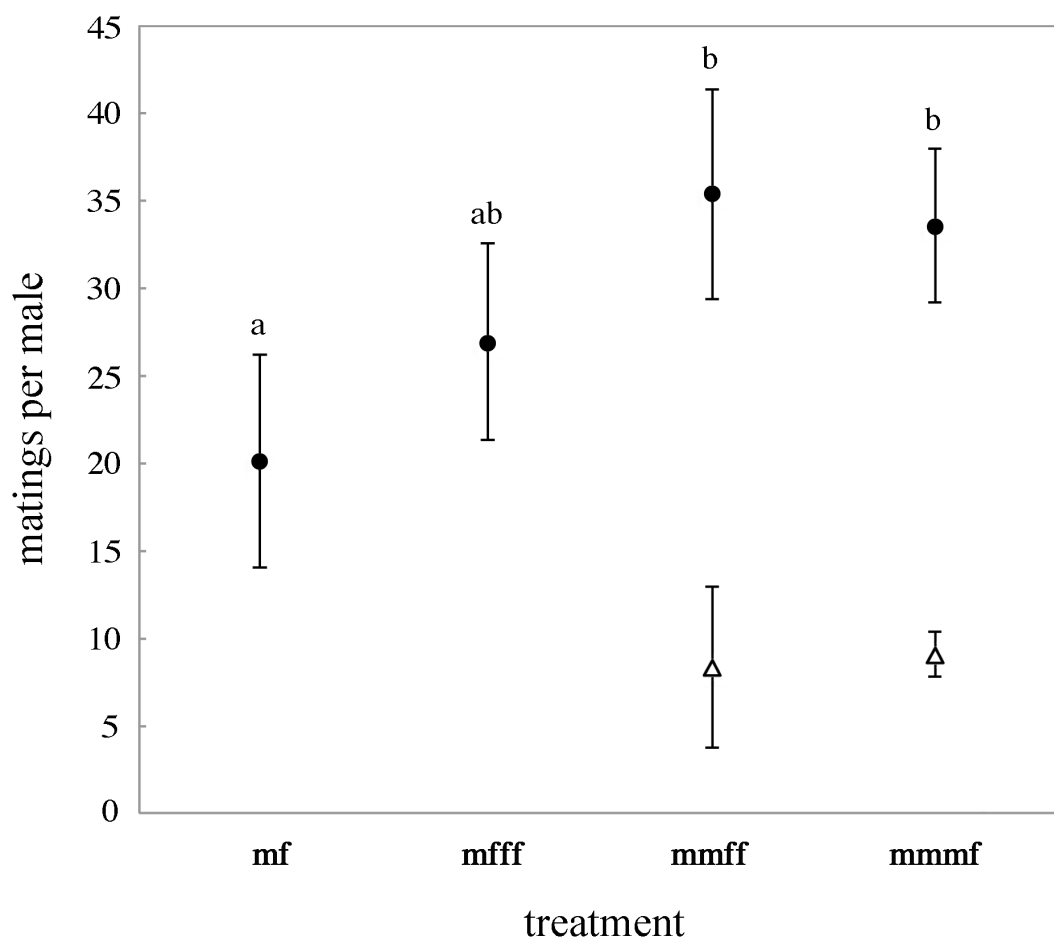
Figure 4.3: Dominant male matings (mean per male) with dominant female (filled circles) and subordinate male matings with dominant female (open triangles). Lower case letters (a & b) indicate significant differences between treatments for dominant pair matings.

Effects of mating frequency on parental performance:

Increased pre-burial mating frequency extended the latency to carcass burial (log(commence burial) $F_{1,66}$ = 11.952, *p* < 0.001)*.* Increased pre-burial mating frequency also prolongs carcass interment (LM: log(carcass burial), $F_{1,65}$ = 22.602, *p* < 0.0001).

Discussion:

We found males that faced a real threat to paternity assurance (i.e., from satellite males or from pre-mated brood parasitic females) extended the duration of time they remained with their partners and the brood compared to males in monogamous pairs (Fig. 4.1). In burying beetles, dominant males that remain at the carcass with their partner actively participate in caregiving duties (Müller & Eggert 1989). This supports the prediction that increasing paternity assurance may be a benefit of prolonging paternal care. Kvarnemo's (2006) model suggests that male care may evolve when there is a net benefit resulting from the combined sum of three factors: a) gained or lost opportunities for mating, b) increased paternity, and c) improved offspring fitness.

Opportunities for mating:

In many species opportunities for mating are reduced for caregiving males but this is not always the case. For example, in some nest-building fish species, males caring for eggs are preferred by spawning females (Ridley & Rechten 1981; Forsgren, Karlsson & Kvarnemo 1996; Alonzo 2008). In burying beetles reproductive success is resource limited rather than mate limited so any trade off between lost mating

opportunities and paternal care may take a different form involving the future likelihood that a carcass is located.

Increased paternity:

It has been established that in *N. vespilloides* sperm precedence is not achieved by the last male to mate, rather it is an increasing function of repeated and consecutive male mating; a male may have to mate seven times even to gain 50% paternity when a female has been pre-mated (Müller & Eggert 1989). Therefore, in the female-biased (mfff) treatment (i.e., where there is no overt male competition) brood parasitic females are cast in the role of competitors for brood parentage not only against the dominant female present but also, through stored sperm, against the dominant male. Müller and Eggert (1989) demonstrated that males on a carcass with exclusive access to a female may achieve paternity above 90%. This is complimentary to the findings presented here and supports the increasing paternity hypothesis: mating frequency was high enough to maximize paternity in treatments with a single (potentially pre-mated) dominant female (Müller & Eggert 1989) and the frequency of mating between dominant individuals increased in the presence of satellite males as would be predicted if males are defending their paternity (Fig. 4.3). Monogamous males also deserted earlier than those that faced male competition or sperm competition thus minimizing lost time for future additional reproductive and/or mating opportunities elsewhere.

Current and future increase in paternity

Kvarnemo (2006) points out that the benefits from increased paternity may be divided into current and future benefits. A study of savannah sparrows, *Passerculus*

sandwichensis, provides an example of a future paternity benefit: male parents that were attentive to a first brood were rewarded by their mates with higher paternity (through higher female fidelity) in their second broods (Freeman-Gallant 1996). In burying beetles a male staying to care might influence his future paternity if females impregnated by him (e.g. while he is engaged in a current breeding bout) find new breeding opportunities. Dominant and brood parasitic females may find further carcasses during the same season and rear broods using the current dominant male's stored sperm. Thus, even a mating after larvae have hatched may increase future paternity for the male when the female rears (or contributes to) another brood, especially when she finds a previously undiscovered carcass.

Males in treatments with satellite male competition mated more frequently with the dominant female than did than did those in treatments with no satellite males present (Fig. 4.3). This fits with the repeated mating mechanism described by Müller and Eggert (1989) by which male burying beetles can maximize their paternity when it is challenged by rivals, either present (satellites) or absent (stored sperm in pre-mated females). A study of wild *N. vespilloides* reported a smaller proportion of larvae sired by absent males (stored sperm) when a male(s) was in attendance at a carcass with a female (Müller *et al.* 2007). Thus, a male mating on a carcass increases his paternity against males employing alternative male tactics (i.e., calling and mating with females without first finding a carcass) and also compared to male competitors immediately present. Another possible selective advantage for males that delay their desertion may lie in ensuring the departing dominant female has freshly replenished sperm stores. Female stored sperm has a limited lifespan, starting to become unviable after three weeks (Eggert 1992). By remaining to secure a late mating

towards the end of a reproductive bout, a male's future reproductive might benefit should the female successfully locate another carcass. Mating rates for dominant females after larvae hatched were relatively low compared to pre-natal rates, but nevertheless averaged 3.28 ± 0.67. These matings could have little or no current benefit to either sex.

Previous laboratory studies report dominant males mated with mean frequencies of 79 times in two days (Müller & Eggert 1989) and as much as 100 times in 24 hours (Pettinger *et al.* 2011). In contrast, the mean mating frequency we recorded between dominants, between their first contact with each other and interment of the carcass (a mean duration of 25 hours 8 minutes ± 2 hours 15 minutes), was 15.1 ± 1.53 times. This difference may be attributable to the naturalistic design we employed allowing beetles to leave and re-enter the central area of the arena at will, and permitting dominants to bury the carcass and engage in normal reproductive behaviour, which contrasts with previous lab study setups. The dominant male invariably copulated with the dominant female whenever a satellite male was detected, whether or not a successful satellite mating had occurred. This response to direct contact with competition was sex ratio-dependent not density-dependent: the dominant female in the female-biased treatment (mfff) suffered no significant increase in mating herself when the male encountered extra female competition (Fig. 4.3).

An alternative explanation for the early desertion seen in males from monogamous pairs might be that there is a lower likelihood of late incursion and brood 'takeover' by competitors perhaps involving killing and substitution of larvae (Scott & Gladstein

1993). However, if risk of brood takeover was an important factor here, males in both treatments without male competitors (i.e., mf and mfff) would be expected to desert early because the brood will be comprised of their offspring whether or not the dominant female is subsequently usurped by female rivals present. Furthermore, in our design the caring female has already established and maintained dominance status against her female competitors (usually by virtue of a size advantage) independent of her male partner.

Offspring benefits

Benefits through increased paternity need not be exclusive of offspring benefits. Kvarnemo (2006) offers the example of nest-building gobies in which males of many species line the inside of their nest (where females lay eggs) with sperm-infused mucus. Therefore his nest both protects the eggs and helps to improve his paternity against rival males (Scaggiante *et al.* 2005; Svensson & Kvarnemo 2005; Svensson & Kvarnemo 2007). One puzzle is that although biparental care is the most common parental association in burying beetles no clear benefit to offspring has been found for biparental care over uniparental care from either sex (Smiseth *et al.* 2005). This is not a prerequisite of Kvarnemo's (2006) hypothesis that proposes evolution of male care without offspring benefit as long as a net benefit to males exists through increased paternity and or mating opportunities. We found no relationship between male duration of care and brood mass but rather, variation in brood mass was related to the competitive treatment.

Parental conflict and increasing paternity

There was a significant effect of the body size difference between dominant beetles on brood mass that may reflect an uneasy coercive tension between caring partners. Increased mating frequency might be beneficial for males when there is male competition (protecting paternity), however multiple mating can be energetically costly and takes time that could be used elsewhere (Chapman *et al.* 1995; Arnqvist *et al.* 2000; Arnqvist & Rowe 2005). Increased time and energy spent in mating (and resisting mating attempts) and resource defence are likely to have different impacts on each partner depending on the sex of the competition introducing conflict between parents (Parker 2006). There is evidence from the closely related *N. defodiens* that in some circumstances (e.g., when the carcass is large enough to support a larger brood than can be produced by a single female) female burying beetles try physically to suppress male attempts to call extra females (Eggert & Sakaluk 1995). Female coercion in male behavioural decisions could be facilitated by a positive size advantage leading to female coercion in early male desertion and/or preference for smaller males. Although we found a positive effect on brood mass related to such a size differential between parents, the largest individual beetles usually prevail in intrasexual contests (Lee *et al.* 2013) so there is potential for size difference between dominant pairs to be correlated with treatment in our design. Thus, these effects of size differences within dominant pairs will have to be independently tested in the future.

Increasing paternity: increased risk

Carcasses are valuable and when exposed in the wild, before burial, are particularly vulnerable to discovery by conspecifics, congenerics and scavengers (especially

slugs, *Arion* sp. in our wild study population, PEH unpublished data). We found a strong negative correlation between mating frequency and the initiation of carcass burial and the time taken for actual interment. It is not clear whether this occurred because males were distracted from helping with carcass interment or because dominant females suffered interference from the intrusions of dominant males eager to upregulate their mating frequency. Nevertheless this delay represents a potentially serious cost to each dominant parent increasing the risk of usurpation by a larger same-sex competitor, or to both parents if the carcass is detected and taken over by congeneric competitors or scavengers (Scott 1990; Müller, Eggert & Sakaluk 1998)

Female duration of care

Female early desertion might have been predicted when there were cues indicating a low proportion of brood parentage for the female (i.e., the female-biased treatment). A well-studied example is penduline tits, *Remiz pendulinus*, where males or females may be impelled to provide uniparental care for a brood by the early desertion of their partner but roughly one third of broods perish due to desertion by both parents (van Dijk *et al.* 2012). We found no evidence that variation in parentage assurance determined the sex that deserted first (in fact dominant females rarely deserted until larvae had finished their development and dispersed). Male burying beetles are capable of caring uniparentally and selection for this ability requires an explanation (Trumbo 1991; Rauter & Moore 2004). However, here we controlled for carcass size and there is evidence from studies of a congeneric species, *N. defodiens*, that carcass size might influence the direction of intersexual parental benefit: large carcasses, beyond the size a single female can populate optimally with larvae induce males to stay and call extra females (Trumbo & Eggert 1994; Eggert &

Sakaluk 1995). Thus, a hypothesis that needs testing is that in this case the dominant female might desert broods early (i.e., on large carcases with female-female competition) leaving a dominant male to care alone for a large brood of mixed maternal origin. Male prolonged attendance at a brood as a response to threats to his paternity may alter the balance of costs and benefits of his providing actual direct care for offspring while he is present. He has paid the potential cost of lost time in the mating pool (mitigated by opportunities to mate with brood-parasitic females); the difference in additional energetic cost between caring for offspring, or not, may be small.

Conclusions:

Male *N. vespilloides* parents responded to manipulated variation in the local competitive social environment when cues indicated reduced paternity assurance by increasing their mating frequency. When confronted with evidence of threats to their paternity caregiving males also increased the duration of time they stayed with their partner performing postnatal caring duties. These threats to paternity might come from active competition from additional males but also from females with stored sperm from other males. Males in monogamous partnerships (i.e., with no extra-pair competition so confidence in paternity is predicted to be highest) exhibited the shortest duration of care. Collectively, results support the hypothesis that when paternity is threatened, males may prolong the time they spend with their partner and the brood when by doing so they can benefit from increasing their paternity. This may occur despite potential costs such as extending the current reproductive bout and reducing the mass of the brood. Variation in the competitive social environment is likely to have a role in shaping the evolution of patterns of parental investment

between sexes by mediating the balance between sexual selection (e.g., males increasing mating success) and natural selection (e.g., male care contributing to offspring fitness) in this and other species.

References:

Alonzo, S.H. (2008) Female mate choice copying affects sexual selection in wild populations of the ocellated wrasse. *Animal Behaviour,* **75,** 1715-1723.

Alonzo, S.H. & Klug, H. (2012) Paternity, maternity, and parental care. *The evolution of parental care* (eds N.J. Royle, P.T. Smiseth & M. Kölliker), pp. 189-205. Oxford University Press, Oxford.

Arnqvist, G., Edvardsson, M., Friberg, U. & Nilsson, T. (2000) Sexual conflict promotes speciation in insects. *Proceedings of the National Academy of Sciences,* **97,** 10460-10464.

Arnqvist, G. & Rowe, L. (2005) *Sexual Conflict*. Princeton University Press, Princeton.

Benowitz, K.M., Head, M.L., Williams, C.A., Moore, A.J. & Royle, N.J. (2013) Male age mediates reproductive investment and response to paternity assurance. *Proceedings of the Royal Society B: Biological Sciences,* **280,** 20131667.

Chapman, T., Liddle, L.F., Kalb, J.M., Wolfner, M.F. & Partridge, L. (1995) Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature,* **373,** 241-244.

Clutton-Brock, T.H. (1991) *The evolution of parental care*. Princeton University Press, Princeton.

Crawley, M.J. (2007) *The R book*. Wiley and Sons, Chichester.

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

Eggert, A.K., Reinking, M. & Müller, J.K. (1998) Parental care improves offspring survival and growth in burying beetles. *Animal Behaviour,* **55,** 97-107.

Eggert, A.K. & Sakaluk, S.K. (1995) Female-coerced monogamy in burying beetles. *Behavioral Ecology and Sociobiology,* **37,** 147-153.

Forsgren, E., Karlsson, A. & Kvarnemo, C. (1996) Female sand gobies gain direct benefits by choosing males with eggs in their nests. *Behavioral Ecology and Sociobiology,* **39,** 91-96.

Freeman-Gallant, C.R. (1996) DNA fingerprinting reveals female preference for male parental care in savannah sparrows. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* **263,** 157-160.

Griffin, A.S., Alonzo, S.H. & Cornwallis, C.K. (2013) Why do cuckolded males provide paternal care? *PLoS biology,* **11,** e1001520.

Gross, M.R. (2005) The evolution of parental care. *The Quarterly review of biology,* **80,** 37-45.

Harrison, F., Barta, Z., Cuthill, I. & Székely, T. (2009) How is sexual conflict over parental care resolved? A meta-analysis. *Journal of Evolutionary Biology,* **22,** 1800-1812.

Hartley, I.R., Davies, N.B., Hatchwell, B.J., Desrochers, A., Nebel, D. & Burke, T. (1995) The polygynandrous mating system of the alpine accentor, *Prunella collaris.* II. Multiple paternity and parental effort. *Animal Behaviour,* **49,** 789-803.

Head, M.L., Berry, L.K., Royle, N.J. & Moore, A.J. (2012) Paternal Care: Direct and indirect genetic effects of fathers on offspring performance. *Evolution,* **66,** 3570-3581.

Hopwood, P.E., Moore, A.J. & Royle, N.J. (2013) Nutrition during sexual maturation affects competitive ability but not reproductive productivity in burying beetles. *Functional Ecology,* **27,** 1350-1357.

Houston, A.I. (1995) Parental effort and paternity. *Animal Behaviour,* **50,** 1635-1644.

Hunt, J. & Simmons, L.W. (2002) Confidence of paternity and paternal care: covariation revealed through the experimental manipulation of the mating system in the beetle *Onthophagus taurus*. *Journal of Evolutionary Biology,* **15,** 784-795.

Kahn, A.T., Schwanz, L.E. & Kokko, H. (2013) Paternity protection can provide a kick-start for the evolution of male-only parental care. *Evolution,* **67,** 2207-2217.

Kokko, H. & Jennions, M. (2008) Parental investment, sexual selection and sex ratios. *Journal of Evolutionary Biology,* **21,** 919-948.

Kokko, H. & Jennions, M. (2012) Sex differences in parental care. *The evolution of parental care* (eds N. Royle, P. Smiseth & M. Kölliker), pp. 101-116. Oxford University Press, Oxford.

Kvarnemo, C. (2006) Evolution and maintenance of male care: is increased paternity a neglected benefit of care? *Behavioral Ecology,* **17,** 144-148.

Lee, V.E., Head, M.L., Carter, M.J. & Royle, N.J. (2013) Effects of age and experience on contest behaviour in the burying beetle, *Nicrophorus vespilloides*. *Behavioral Ecology,* **25,** 172-179.

Lessells, C.M. (2012) Sexual conflict. *The evolution of parental care* (eds N.J. Royle, P.T. Smiseth & M. Kölliker), pp. 150-170. Oxford University Press, Oxford.

McNamara, J.M., Houston, A.I., Barta, Z. & Osorno, J.-L. (2003) Should young ever be better off with one parent than with two? *Behavioral Ecology,* **14,** 301-310.

Müller, J., Eggert, A. & Sakaluk, S. (1998) Carcass maintenance and biparental brood care in burying beetles: are males redundant? *Ecological Entomology,* **23,** 195-200.

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

Müller, J.K. & Eggert, A.K. (1989) Paternity assurance by "helpful" males: adaptations to sperm competition in burying beetles. *Behavioral Ecology and Sociobiology,* **24,** 245-249.

Müller, J.K., Eggert, A.K. & Dressel, J. (1990) Intraspecific brood parasitism in the burying beetle, *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Animal Behaviour,* **40,** 491-499.

Neff, B.D. (2003) Decisions about parental care in response to perceived paternity. *Nature,* **422,** 716-719.

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

Parker, G.A. (2006) Sexual conflict over mating and fertilization: an overview. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **361,** 235-259.

Parker, G.A., Royle, N.J. & Hartley, I.R. (2002) Intrafamilial conflict and parental investment: a synthesis. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences,* **357,** 295-307.

Pettinger, A.M., Steiger, S., Möller, J.K., Sakaluk, S.K. & Eggert, A.K. (2011) Dominance status and carcass availability affect the outcome of sperm competition in burying beetles. *Behavioral Ecology,* **22,** 1079-1087.

Pukowski, E. (1933) Ökologische untersuchungen an Necrophorus F. *Zoomorphology,* **27,** 518-586.

R Development Core Team (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org.

Rauter, C.M. & Moore, A.J. (2004) Time constraints and trade-offs among parental care behaviours: effects of brood size, sex and loss of mate. *Animal Behaviour,* **68,** 695-702.

Ridley, M. & Rechten, C. (1981) Female sticklebacks prefer to spawn with males whose nests contain eggs. *Behaviour,* **76,** 152-161.

Royle, N.J., Hartley, I.R. & Parker, G.A. (2002) Sexual conflict reduces offspring fitness in zebra finches. *Nature,* **416,** 733-736.

Royle, N.J., Smiseth, P.T. & Kölliker, M. (2012) *The evolution of parental care,* 1st Edition. Oxford University Press, Oxford.

Scaggiante, M., Rasotto, M.B., Romualdi, C. & Pilastro, A. (2005) Territorial male gobies respond aggressively to sneakers but do not adjust their sperm expenditure. *Behavioral Ecology,* **16,** 1001-1007.

Scott, M.P. (1989) Male parental care and reproductive success in the burying beetle, *Nicrophorus orbicollis. Journal of Insect Behavior,* **2,** 133-137.

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

Scott, M.P. (1998a) The ecology and behavior of burying beetles. *Annual Review of Entomology,* **43,** 595-618.

Scott, M.P. (1998b) Facultative adjustment of the duration of parental care by burying beetles. *Journal of Insect Behavior,* **11,** 597-603.

Scott, M.P. & Gladstein, D.S. (1993) Calculating males? An empirical and theoretical examination of the duration of paternal care in burying beetles. *Evolutionary Ecology,* **7,** 362-378.

Smiseth, P.T., Darwell, C.T. & Moore, A.J. (2003) Partial begging: an empirical model for the early evolution of offspring signalling. *Proceedings of the Royal Society B,* **270,** 1773-1777.

Smiseth, P.T., Dawson, C., Varley, E. & Moore, A.J. (2005) How do caring parents respond to mate loss? Differential response by males and females. *Animal Behaviour,* **69,** 551-559.

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

Svensson, O. & Kvarnemo, C. (2005) The importance of sperm competition risk and nest appearance for male behavior and female choice in the sand goby, *Pomatoschistus minutus*. *Behavioral Ecology,* **16,** 1042-1048.

Svensson, O. & Kvarnemo, C. (2007) Parasitic spawning in sand gobies: an experimental assessment of nest-opening size, sneaker male cues, paternity, and filial cannibalism. *Behavioral Ecology,* **18,** 410-419.

Trivers, R.L. (1972) *Parental investment and sexual selection*. Aldine, Chicago.

Trumbo, S.T. (1991) Reproductive benefits and the duration of paternal care in a

biparental burying beetle, *Necrophorus orbicollis*. *Behaviour,* **117,** 82-105.

Trumbo, S.T. & Eggert, A.K. (1994) Beyond monogamy: territory quality influences

sexual advertisement in male burying beetles. *Animal Behaviour,* **48,** 1043-

1047.

van Dijk, R.E., Székely, T., Komdeur, J., Pogány, Á., Fawcett, T.W. & Weissing, F.J.

(2012) Individual variation and the resolution of conflict over parental care in

penduline tits. *Proceedings of the Royal Society B: Biological Sciences,* **279,**

1927-1936.

Whittingham, L.A., Taylor, P.D. & Robertson, R.J. (1992) Confidence of paternity and

male parental care. *American Naturalist,* **139,** 1115-1125.

Chapter 5:

Paternal duties versus mating opportunities: do sex ratio cues affect the duration of male burying beetle parental care?

Summary:

Male parents face a choice: should they invest more in caring for current offspring or devote their efforts to attempting to mate with other partners. The most profitable course will depend on the intensity of competition for mates, which is likely to vary with the population sex ratio. If male parents have a cue providing information about sex ratio they have the opportunity to base a decision to care or desert on perceived mating opportunities available elsewhere. However, an individual's decision may also depend on his relative prowess in competing for mates. We tested the prediction that parental desertion time in male burying beetles, *N. vespilloides*, would be affected by population sex ratio cues by rearing males, post-eclosion, in groups that differed in sex ratio. Focal males were subsequently provided with a carcass (breeding resource) in the wild and filmed. We found no effect of rearing sex ratio on parental desertion decisions, but smaller males were more successful at attracting females in the wild than were larger males and this increased the relative success of smaller males in securing monogamous breeding associations. Smaller males thus avoided competitive male-male encounters, more often than larger males, gaining exclusive access to both female and breeding resource.

Introduction:

In species that provide parental care, although there is wide variation among taxa, females often provide the bulk of care while males tend to spend more time seeking

out further mating opportunities (Kokko & Jennions 2012). One factor that may influence the pattern of these sex role differences is the ratio of females to males in the population. Most sexual species produce females and males in similar numbers but there are often biases in the ratio of available, receptive mating partners: the operational sex ratio (OSR) (Emlen & Oring 1977; Kvarnemo & Ahnesjö 2002). OSR bias, by differentially affecting how difficult it is to find a mate, can influence the intensity of mating competition (Clutton-Brock & Vincent 1991; Andersson 1994). This could alter the balance between the cost and benefit of caring for offspring, versus trying to secure more mating partners (Fromhage, McNamara & Houston 2007; Kokko & Jennions 2012).

Theoretical models incorporating the OSR have produced differing explanations for sex specific patterns of parental investment and mating competition. One prediction is that with fewer receptive females than males in a population, males should be selected to invest less in parental care, maximising the time available to pursue scarce additional mating opportunities (Trivers 1972). In this case, the strength of sexual selection for competitive ability is predicted to increase due to intensified competition for mating among the excess of males (Trivers 1972; Emlen & Oring 1977; Kvarnemo & Ahnesjo 1996). However, recent models formalise an alternative pattern where selection operates to increase parental investment in the over-represented sex because these good parents will benefit from maximising their returns from actualised reproductive events (Kokko & Jennions 2008; Kokko & Jennions 2012). Although the models differ in their predictions about the effects of OSR one common assumption is that the sex ratio is constant (Kokko, Klug & Jennions 2012). However, it is not known how parents might adjust their investment

in species that experience dynamic changes in the direction or magnitude of sex ratio bias between reproductive events. Species that experience variation in the sex ratio within and among reproductive seasons provide an opportunity to test the core predictions of models by examining the effects of manipulated sex ratio cues on caring behaviour and its benefits.

Interactions between traits that influence fitness have evolved in the context of ecological environments that link fitness and phenotypic variation (Wade & Kalisz 1990). Although environmental agents of selection (the causes of adaptive evolution) can be difficult to identify or quantify (MacColl 2011), but there may be ecological factors affecting parental investment decisions that can be generalised among species. For example, breeding resources other than mates may be the limiting factor for reproductive success shifting parental investment decisions away from model predictions (Kvarnemo & Ahnesjo 1996). Almada *et al.* (1995) made a comparative observational study of blennies, *Salaria pavo*, in which the usual sex roles in this species were reversed in a population with exceptionally scarce nest sites. Instead of the usual pattern of males courting females, females courted males and both sexes (rather than exclusively males) engaged in intrasexual aggression. Breeding resource unpredictability may alter the effect of OSR in different ways than does resource scarcity. For example, opportunistic reproduction dependent upon unpredictable resource availability in some species may select for facultative plastic responses to local conditions because there is no single optimal strategy (Shine & Brown 2008). One way to study the interactions between ecological factors (e.g., sex ratio, resource availability or population density) and reproductive behaviour, would be to manipulate cues to potential environmental conditions in the laboratory but

then to observe the effect on treated individuals interacting with wild individuals in a naturalistic setting.

Burying beetles, *Nicrophorus sp.* are ideal study species to take such an approach. They rely wholly on a breeding resource (small vertebrate carcasses) that is both scarce and unpredictable, although mating itself may occur in the presence or absence of such a breeding resource (Eggert & Müller 1997). The relationship between mating success and reproductive success is defined by the availability of carcasses because a carcass is necessary to rear offspring (Eggert & Müller 1997). This in turn is mediated by variation in individual success (within both sexes) in locating and competing for carcasses (Müller & Eggert 1987). For male burying beetles the relationship between mating success and reproductive success differs between two mating contexts that correspond to alternative mating strategies (Müller *et al.* 2007). Firstly, males may call (by emitting pheromones), and mate with females despite the absence of a carcass. In this context, the number of mating partners is likely to be important because a proportion of these mated females may subsequently locate a carcass and reproduce using stored sperm (Müller *et al.* 2007). Secondly, a male may benefit from increased paternity by securing dominant (or exclusive) male status on a carcass (House, Hunt & Moore 2007; Müller *et al.* 2007). In this second context he has the opportunity to maximize the precedence of his sperm against that of competitors (present or absent) (Müller & Eggert 1989; chapter 4). Because both sexes search for suitable breeding carcasses (chapter 6) the local sex ratio can be biased in either direction and the intensity of competition during a single breeding attempt depends on the number and identity of beetles attracted to a single carcass. However, despite the limitation of carcass availability

and the ability of both sexes to provide effective postnatal care (Ward, Cotter &

Kilner 2009), burying beetles maintain a female-biased care pattern. The majority of

breeding events appear to be biparental (in which males invariably desert first), the

next most common breeding association is female uniparental care and male

uniparental postnatal care is the least common (Eggert & Müller 1989; Eggert 1992;

Müller *et al.* 2007).

We found previously that the social environment at a carcass influenced the

desertion behaviour of male parents (chapter 4). Males in monogamous

(uncontested) partnerships deserted earlier and mated less frequently than did

males that faced competitors at the carcass (chapter 4). This may have been due to

males protecting their paternity against competition, but an alternative (non mutually

exclusive) hypothesis for variation in male care is that relative marginal gain between

deserting males and caring males may differ according to male competitive ability

(relative size) and OSR bias. Although each female with whom a male mates has a

chance of finding a carcass and reproducing alone, large males may have an

additional advantage over small males because they are more likely to achieve

dominant status in future reproductive contests over carcasses. If OSR bias at the

population level influences the social environment at carcass level, encounter rates

experienced before a carcass is located might provide males with a cue to likely

population sex-ratio (and/or density) on which to base future desertion decisions.

The effects of an unpredictable sex ratio on mating competition and parental

investment are not known but one possible outcome is selection for plastic

behavioural responses. We tested individual behavioural responses induced by

experimental manipulation of sex ratio cues in a natural setting. Specifically we wished to find out how male competitive ability (body size) might interact with sex ratio cues to influence male parental care. We reared and kept mature male burying beetles, *N. vespilloides*, in four different treatment groups providing different sex-ratio cues: groups consisted of an experimental male and either two additional males and a female (male bias); three females (female bias); two females and one additional male (equal sex ratio); or an experimental male alone (no cue). Experimental male beetles were removed after two weeks, measured and weighed, then marked and released in the wild onto carcasses. Their behaviour and that of subsequent wild arrivals was recorded to provide comparisons between the manipulated early sex ratio cues versus realised competitive environment at a carcass. In order to quantify the proportion of natural breeding associations that involve a lone male locating a carcass and calling for a partner (i.e., to verify our experimental scenario) we conducted a second experiment in which we placed carcasses in the field without beetles and recorded the order, sex and/body size of wild beetles that arrived.

Based on findings described in chapter 4 we predicted that males experiencing a male sex ratio bias in early adult life would prolong parental care compared to those experiencing a female sex ratio bias, standing to benefit from extended parental care by maximising the return (i.e., protecting paternity) from their current brood. We also predicted large males would desert broods before small males. This is because contest success (resource-holding potential) is positively correlated with body size (Otronen 1988; Lee *et al.* 2013) so larger males have a greater likelihood of

dominating future contested reproductive opportunities altering the balance of current cost and future benefits in favour of earlier desertion.

Methods:

Experiment 1:

To minimize potential confounding effects both of unintentional laboratory selection and experiential variation among wild individuals we generated F1 stock for use in this experiment. 669 wild beetles were caught in funnel-type bottle traps baited with rotten salmon on a weekly basis throughout the spring and summer of 2012. Captured beetles were relieved of phoretic mites (by blowing with air), fed on decapitated mealworms *ad libitum* for one week (in case trap-caught beetles were immature youngsters). Within the pool of beetles captured each week pairs were randomly assigned a mouse carcass on which to breed. In total 307 of the resulting F1 progeny were randomised within capture weeks and assigned to one of four treatment groups producing 91 experimental males that had experienced a manipulated sex ratio cue (i.e., one male from each group).

Sex ratio cue manipulation:

The four sex ratio cue treatment groups consisted of 1) female bias: three females and one male (mfff); 2) male bias: one female and three males (mmmf); 3) no sex-bias: two females and two males (mmff); 4) absence of cue: a single virgin male (m). These groups of beetles were housed together for two weeks from eclosion in plastic boxes 32 × 18 × 12cm with 3cm moist compost and egg-cartons to provide three-dimensional structure allowing places to hide and calling platforms. Boxes were kept on shelves outside the laboratory to minimise differences in seasonal photoperiod and climactic experience between experimental males and the wild beetles with

whom they would interact (the field site is 2km from the laboratory). Experimental

groups were fed decapitated mealworms at the rate of two per individual twice

weekly. All individuals were initially weighed, measured and marked (using Indian ink

dots on elytra, see chapter 4) and after two weeks a single male was removed from

each container and used as the focal experimental beetle.

Nicrocosms

400mm lengths of black PVC-U 110mm Ø pipes ('Nicrocosms') were buried vertically

up to 100mm with three 40mm Ø exit and entry ports surrounding the structure

positioned at ground level (Fig 4.1b). These ports were extended with 40mm Ø PVC-

U pipe to protrude 100mm and reduce the ingress of light to the interior space. Six

further ventilation holes (10mm Ø) were drilled towards the top of each Nicrocosm.

Infra-red surveillance cameras (N08CX night vision CCTV camera) using motion

detecting software (AVerMedia NV6240 Express, DVR version 7.7.0.0007;

www.avermedia-dvrs.com) were positioned within to capture beetle activity around a

mouse carcass that was positioned inside and a small inverted red polypropylene

bucket protected the interior space and from rain and light (Fig 4.1a).

The experimental site is mixed deciduous woodland with populations of congeneric

burying beetles: *N. vespilloides, N. humator, N. investigator, N. interruptus* and *N.

vespillo* in descending order of frequency caught in traps (PEH 2011; 2012; 2013

unpublished data). Experimental male beetles were placed in Nicrocosms in series

(but chosen haphazardly with regard to treatment group) from early July to mid

September 2012, spaced at least 20m apart, with six or fewer replicates running

concurrently in a study area of approximately 0.5ha (coord: N50° 11' 42", W5° 07'

51"). Each Nicrocosm contained a 24 hour thawed mouse carcass with no artificial

restriction imposed on carcass burial depth. This protocol allowed observation of

above ground activities such as fights and outcomes, desertion and arrivals.

Moreover, it permitted evaluation of rates of takeover by other beetles because

although beetles were free to conceal carcasses, new arrivals were obvious when

they arrived above ground. Allowing unrestricted carcass burial had the

inconvenient, but anticipated consequence that qualitative records of active parental

care were rarely obtainable. Single, marked experimental males were introduced to

Nicrocosms at approximately midday whereupon they invariably took cover in the

soil substrate before emerging to explore later in the day during the species normal

activity period (usually about 16:00). Each replicate ran until desertion of the

experimental male was witnessed (assessed as the male witnessed leaving the

Nicrocosm by a port and absent for >6 hours). Males and females that had been

engaged in parental care often unfurled their wings prior to leaving voluntarily,

perhaps in readiness for flight; this behaviour was not witnessed in beetles that were

evicted forcibly by others. Of a total of 91 experimental males placed in Nicrocosms

in experiment 1, 76 were witnessed calling before the arrival of wild beetle(s) and

used in analyses of arrival times and sex of wild beetles. We find un-utilized

carcasses are devoured by slugs (predominantly *Arion* spp.) and depending on size,

become unusable for breeding by burying beetles after two or three nights. In 11

instances (out of 91) no wild beetle(s) arrived after two successive evenings of males

calling and these carcasses were lost to slugs. 3 further replicates failed because the

experimental male deserted without establishing contact with the carcass. One

further male called but the times of subsequent arrivals were not clear from footage

obtained. No additional burying beetles were witnessed arriving after carcass burial

was complete during this experiment and none of the four congeneric species was

seen in the nicrocosms. Congeneric species in the UK are easy to distinguish visually because only *N. vespilloides* has black (rather than red) antennae tips.
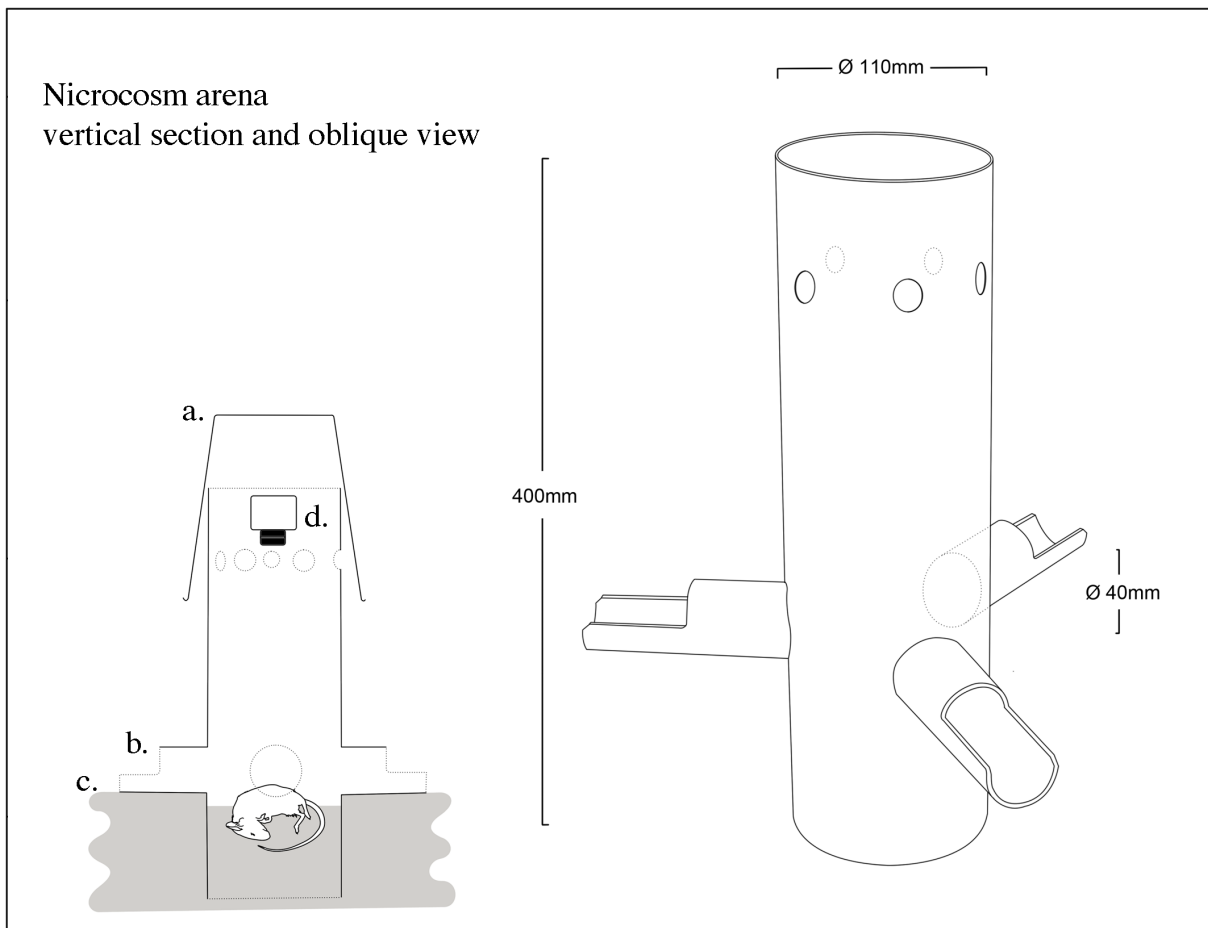
Figure 5.1: Nicrocosm arena for filming behaviour of wild beetles showing, a. inverted red bucket; b. exit/entry ports; c. ground level; d. camera placement. Oblique view indicates dimensions.

Behavioural observations:

We defined first contact with the carcass as the time of first exploration of the carcass surface by a male (rather than physical contact incidental to hiding or running past). Male calling was assumed when males ceased walking and adopted a characteristic *sterzeln* posture: tail-up, head-down with abdominal segments extended (Pukowski 1933; Müller & Eggert 1987). We measured time until male desertion as total pre- and postnatal attendance measured in dominant experimental males as time between arrival of the first wild female, and the time when the male

left. Only experimental males that maintained a dominant (caregiving) position were included in the analysis of desertion because usurped males do not remain to provide care for the brood. We exhumed carcasses after dominant male desertion to confirm his absence, to corroborate number and sex of any remaining beetles and to ensure that the reproductive bout was successful (i.e., larvae were produced).

Experiment 2:

In order to determine the proportion of males and females that initially locate carcasses we placed mouse carcasses in Nicrocosms at the field site in the summer of 2013. We used a similar spatial placement protocol as described above but reviewed video footage each morning to determine the carcasses that had attracted beetles. Utilised carcasses were disinterred the day after burial and the beetles sexed, weighed and measured and categorised by the order that they arrived by matching beetle size, markings and mating and/or fighting behaviour witnessed through video footage. Carcases remaining unclaimed and unburied were discarded after 3 days and replacements sited in different locations.

Statistics:

All analyses were performed using 'R' version 2.14.1 (R Development Core Team 2011). Male desertion (i.e., the duration of his biparental association with his partner) had a non-normal distribution unresponsive to transformation so Kruskal-Wallace non-parametric analyses were used. Sex of first arrival was analysed using a generalised linear model with a binomial error structure with treatment, male size (males were categorised as small or large according to whether their pronotal width fell above or below the median for experimental males) and the interaction between them as fixed effects with the minimal adequate model determined through stepwise

model simplification. Fisher's exact tests were used to analyse counts of focal males in different categories of body size and breeding association (i.e., counts of those encountering male-male competitive associations and counts of breeding associations involving female-female competition). We also tested for a possible confounding male response to the sex ratio treatments: a treatment effect on the calling behaviour of experimental males might have affected the number or sex of beetles arriving at a carcass. Therefore, the time between a male's first contact with a carcass and commencing calling (measured in minutes) was log transformed to normalise the distribution and analysed using a linear model with treatment, male size and the interaction between them as fixed effects.

For experiment 2, the sex ratio of first arrivals was tested against an expected 0.5 using χ^2 goodness of fit test. We further categorised beetles by sex and according to whether they were first arrivals or subsequent arrivals (i.e., either to a male already arrived and calling or to a carcass without a male present). Mean pronotum width in millimetres among these four groups was analysed using ANOVA with *post hoc* Tukey's honestly significant difference. Unless stated, means are presented ± 1 standard error throughout.

Results:

Experiment 1:

Time until desertion for experimental males that maintained their dominance was 249 ± 52 hours (mean ± SD), and this did not differ significantly among treatments (Kruskal-Wallace rank sum, χ^2_3 = 4.052, *p* = 0.256), nor, according to a separate test, between males split into large and small size categories at the median size (Kruskal-Wallace rank sum, χ^2_1 = 2.822, *p* = 0.093). Male care duration did not differ

between those in monogamous pairs and those in associations with male and/or female extra-pair competitors, i.e., the immediate social environment at the carcass (Kruskal-Wallace rank sum, χ^2_1 = 0.525, p = 0.469).

Experimental males placed on carcasses in the field did not differ in size among the four treatments, (LM pronotum, $F_{3,87}$ = 0.971 p = 0.410), grand mean ± SD = 4.84 ± 0.35mm. There was wide variation among males in the length of delay before calling: on average males commenced calling 81 ± 82 minutes (mean ± SD), after contact with a carcass with the mean time of day males began calling being 17:38). This delay between carcass contact and commencing calling did not differ among treatments (LM, treatment, $F_{3,74}$ = 1.801 p = 0.658) nor between males of different size (male size, $F_{1,73}$ = 1.388 p = 0.268); there was no interaction between treatment and male size (treatment × male size, $F_{3,70}$ = 0.164, p = 0.920).

Male size affected the sex of the first wild arrival: more females than males arrived first to carcasses with small experimental males (male size, χ^2_1 = 4.252, p = 0.039, Fig. 5.2a).
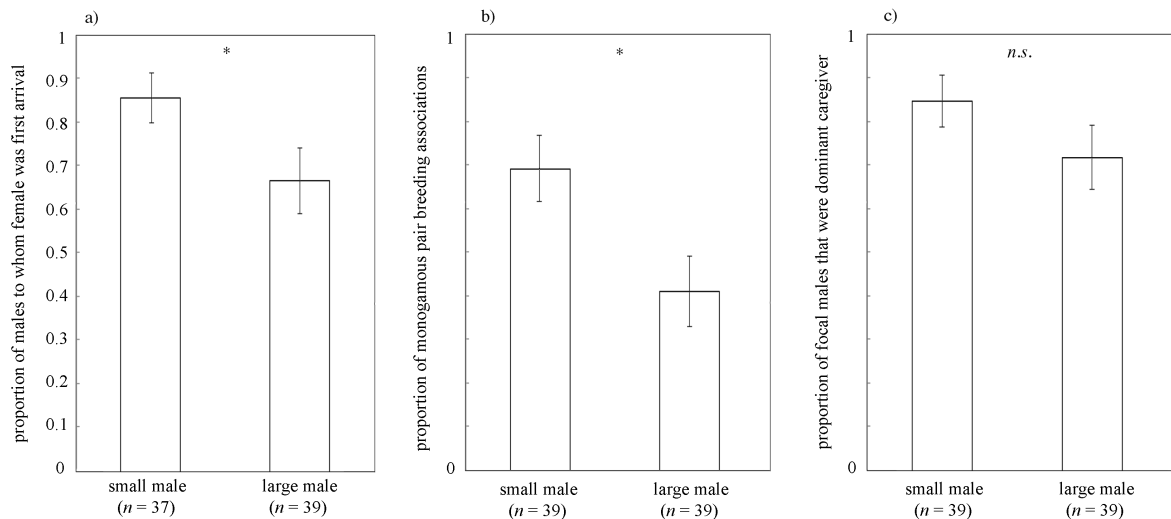
Figure 5.2: a) Proportion of small and of large experimental male beetles calling at a carcass at which the first wild beetle to arrive was female. b) Proportion of small and of large experimental males that experienced uncontested monogamous reproductive bouts (i.e. focal males that bred without encountering extra-pair wild individuals of either sex). c) Proportion of small and of large experimental male beetles that retained dominant status (i.e. they became a caregiving parent). *x* axis categories are males below median size 'small male' and above median size 'large male'. In panel a) data from males not observed calling before the arrival of a wild beetle were excluded. Statistical significance indicated ('*' = *p* < 0.5; '*n.s*'. = *p* > 0.5).

Small experimental males more frequently became partners in uncontested monogamous pairs than at contested carcasses (carcasses with more than two individuals) than did large males (Fisher's exact test; number of small versus large males in monogamous pairs versus contested associations, *n* = 76 *p* = 0.020, Fig. 5.2b and Fig. 5.3a). Small males also experienced less male-male competition than did large males, i.e., related to their disproportionate success in attracting females small males met fewer male rivals (Fisher's exact test; number of small versus large males encountering male rival versus encountering only female(s), *n* = 76 *p* = 0.014, Fig. 5.3b).

17 out of the 76 calling males to whom wild beetles arrived were usurped from a dominant position but overall—although these 17 individuals were likely to have lost to larger opponents—focal male dominant status was not related to body size (GLM, binomial; male size, χ^2_1 = 1.643, p = 0.200, Fig. 5.2c).

Female-female competition (i.e., more than one female arriving to contest dominance at a carcass) was more frequent in breeding associations where there was male-male competition (i.e., there was potentially more than one male contesting and/or calling at a carcass) than with single males (Fisher's exact test; number of breeding associations with >1 female versus 1 female having >1 male versus 1 male n = 76, p < 0.0001, Fig. 5.3c).
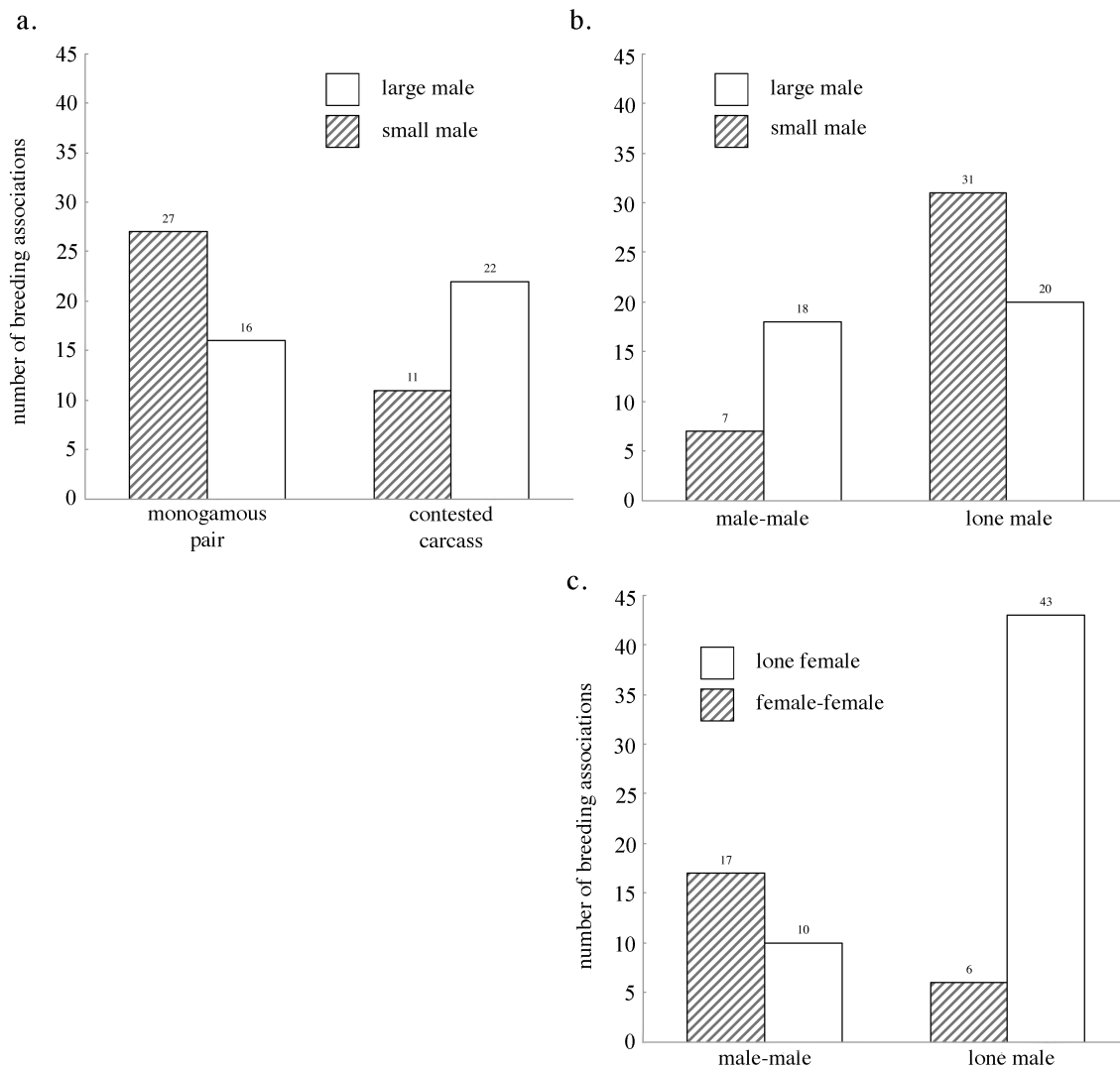
Figure 5.3: Different breeding associations resulting from wild beetles arriving at carcasses originally occupied by experimental male beetles: a) Number of focal male beetles breeding in pairs or as part of a contested carcass (i.e., at least one extra-pair male or female attracted to carcass); b) Number of focal male beetles by size experiencing male-male competition at a carcass; c) Breeding associations with female-female competitive encounters at a carcass related to whether a lone male or more than one male is present at a carcass (i.e., independent of male identity: focal or wild). Numbers above columns indicate numbers of breeding associations recorded (total number = 76).

Experiment 2:

There was no significant deviation from a 1:1 sex ratio in beetles locating carcasses unoccupied by calling males (n = 29 males; 30 females, χ^2_1 = 0.017, p > 0.896).

Males that were the first male to arrive at a carcass (i.e., they couldn't have responded to male pheromones) were smaller than females that arrived at carcasses first but did not differ significantly from males that arrived to carcasses already occupied by a male (ANOVA, size, $F_{3,77}$ = 3.851, p = 0.013, Fig. 5.4).
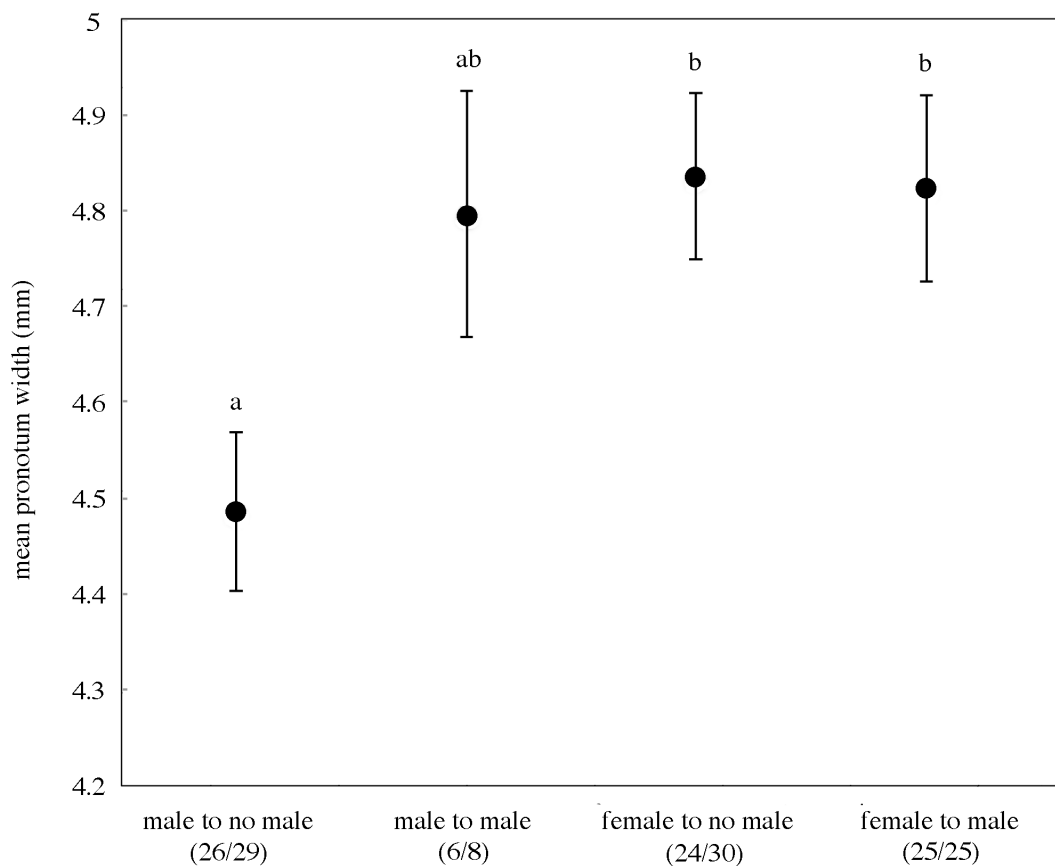
Figure 5.4: Mean size of wild beetles arriving at carcasses placed in the field. Numbers under x axis groups indicate the number of beetles successfully retrieved for analysis and the total number of beetles recorded in each category. Lower case letters over means and standard errors indicate Tukey's honestly significant differences at p <0.05.

Discussion:

Sex ratio treatment and parental investment:

Our first prediction was that male duration of care might be prolonged by a cue, prior to breeding, indicating a male sex ratio bias. This is because extra parental investment in the current realised brood might pay greater dividends (in terms of

improved offspring fitness) than the uncertain pursuit of future reproductive gains (Kokko & Jennions 2008; Kokko & Jennions 2012). However, we found no evidence of differences in male desertion time in relation to sex-ratio cues. Perhaps this is not surprising in hindsight as a reasonable *a priori* prediction was that care decisions should be based on the actual competitive environment faced during each reproductive bout (chapter 4). One reason for this is that the fitness value of additional future mating opportunities depends on carcass availability (necessary to translate matings into material fitness gains) and is also contingent on the context in which matings occur (i.e., with or without a carcass, and if with: as dominant or satellite). Any potential influence of sex ratio bias on trade off decisions between parental investment and improved mating success may be overwhelmed by the effects of these higher-level reproductive contingencies.

In addition to unpredictable breeding resource availability and variation in mating context, the relationship between mating success and fitness in males is mediated by the proportion of carcasses subsequently found by mated single females. Although offspring in a breeding pair are sired overwhelmingly by the resident male(s) (e.g., 89.1% in Müller *et al.* 2007) the proportion of single females breeding alone, using sperm from previous matings, may also be high (e.g., 39% in Eggert 1992). This is supported by the results of experiment 2 where we found no difference in the frequency of males or females discovering carcasses (29 versus 30 respectively). Among these uniparental females, there is potential for previously deserted males to translate off-carcass mating opportunities to fitness gains by increasing effort in calling and mating with females without having found a carcass. Such a trade off, between male parental investment and seeking additional mating opportunities is likely to be affected by the frequency of encounters between

potential mates and competitors but could also depend on differences between males in success of attracting mates (Kokko & Rankin 2006; de Jong *et al.* 2012).

Sexual selection and male strategy:

Very little is known about the strength or importance of sexual selection in burying beetles in the wild but previous studies have posited a fitness advantage for larger than average males due to their competitive prowess (e.g., Creighton 2005; Eggert 1992). Our second prediction was that relative male size might be a factor in male mating success (by virtue of some mating advantage conferred by size), selecting for early desertion of parental duties in specific males that stand to gain more than others from prompt return to the mating pool. Support for this prediction requires evidence that a subset of males actually does secure disproportionate mating success in an ecological context. Although we found no evidence that early parental desertion was correlated with male body size, examination of male size unexpectedly revealed that smaller males were disproportionately successful among males at calling female partners (Fig 4.2a). This had the result that small males were significantly more likely to breed in a socially monogamous pair (Fig 4.2b). We couldn't detect a relationship between male body size and calling behaviour that accounted for small male success in attracting females. One explanation is that wild beetles are responding to variation in the chemical composition of male pheromones. Experimental evidence exists in *N. orbicollis* that females may discriminate between males based on pheromone composition (Beeler, Rauter & Moore 2002). There is also evidence in *N. vespilloides* for wild female preference for smaller calling males based on pheromone discrimination, and laboratory evidence for a female mating preference for the smaller of two males when an immediate choice was available (A. J. Moore, unpublished data). In the cockroach, *Nauphoeta cinerea*, females are

attracted to a pheromone component inversely correlated with male dominance status (Moore *et al.* 2001). Dominant males express high levels of pheromone components (2-methylthiazolidine and 4-ethyl-2-methoxyphenol) associated with competitive success in male-male competition, but relatively low levels of 3-hydroxy-2-butanone which appears to be key to female preference (Moore *et al.* 2001). Females having exercised this choice produced fewer offspring but this was offset by higher offspring quality, avoidance of risks of aggressive male conflict and increased longevity (Moore, Gowaty & Moore 2003).

Laboratory studies of *N. vespilloides* have shown that positive body size differences largely determine dominance status (Bartlett & Ashworth 1988; Otronen 1988; Müller, Eggert & Dressel 1990; Hopwood, Moore & Royle 2013; Lee *et al.* 2013) and therefore reproductive success (Müller *et al.* 2007) on contested carcasses. The conclusions of these studies were based on the syllogism: a) big beetles win contests; b) contest winners have greater reproductive success; c) therefore big beetles have greater reproductive success. In chapter 3 we found that any fitness disadvantage of relatively small beetles due to low likelihood of contest success depends on the social environment they face at carcasses (i.e., the relative size of opponents). We report here that in nature small males effectively avoided direct male-male competitive encounters. This was because when a female arrived first in response to a calling male, the male usually ceased calling, the pair mated, buried the carcass and the likelihood of further arrivals was reduced. Selection may favour large males in context of male-male competitive encounters, but they appear to be selected against by females arriving first to small males who thus avoid contests. Overall this effectively negates the competitive advantage held by large males;

success in securing a breeding resource cannot be predicted *a priori* by size (Fig 4.2c) because small males avoid male-male competition. Males appear to be subject to opposing selection with male-male competition and female mate choice acting antagonistically on male size. Hunt *et al.* (2009) note that it is hard to quantify the combined effects of sexual selection on a targeted trait without detailed knowledge of the direction, strength and interaction of male-male competition and female mate choice. In these beetles the importance of the interaction depends on social context which itself is modified by the mechanisms of sexual selection.

In nature the agents of selection (the reason for correlations between fitness and variation in trait values) are often difficult to identify (Wade & Kalisz 1990; MacColl 2011). In the cerambycid beetle, *Trachyderes mandibularis*, larger males practice a form of resource defence polygyny by successfully defending scarce sites where sap oozes from desert broom, *Baccharis sarothroides* against smaller males (Goldsmith 1987; Goldsmith & Alcock 1993). Large males gain a mating advantage over smaller males when females come to feed at these sites that are defendable by one individual. However when males of the same beetle species attempt to defend saguaro cactus fruits, *Cereus giganteus*, the mating success of smaller males is not compromised because large males cannot monopolise the resource (Goldsmith and Alcock 1992; Goldsmith 1987). Competition for limited breeding resources other than mates is likely to intensify intrasexual competition (Clutton-Brock & Huchard 2013) and in burying beetles carcass availability (relative to population density) is a good candidate as the primary agent of selection on size. When the beetle population is dense compared to availability of suitable breeding carcasses larger beetles might be favoured by a higher frequency of competitive encounters on carcasses which they are more likely to secure. Creighton (2005) found a positive correlation between

population density and size of trapped beetles among different populations of *N. orbicollis.* Further laboratory experiments (Creighton 2005) suggested this effect might be adaptively plastic via parents rearing fewer but larger offspring (i.e., better competitors) when exposed to cues indicating a high density population (but see Steiger *et al.* 2007). Large male burying beetles only benefit, from the competitive advantage conferred by their size, on a contested carcass (chapter 3) while small beetles are more likely to be disadvantaged in direct contests. However, here we present evidence that variation in the social environment seems to be a dynamic function of sexual selection: small male beetles' disproportionate success in attracting females strongly influences the frequency of intrasexual contests.

Female mate choice?

The relationship between male attractiveness and male investment in parental care may depend on whether females choose males on the basis of direct or indirect benefits (Moller & Thornhill 1998). However, in burying beetles postnatal maternal effects strongly influence the size of offspring (Steiger 2013) thus indirect benefits of female mate choice based on male body size are likely to be decoupled. This study suggests that, for males, optimal adult size is determined by the current social environment with which an individual must interact. That social environment, i.e., different combinations of breeding and competitive association, depended on the status and sex of the first beetle to discover a carcass (experiment 1). When a male is the first on the scene he must call for a female. If successful in attracting a female the pair have a reasonable chance of burying their carcass before further challengers arrive. However, if a calling male attracts another male the outcome is more uncertain: a female may coerce a single male into ceasing calling (Eggert &

Sakaluk 1995) but any additional males may continue calling to attract further females. Consequently there is a significant positive association between male-male competition and female-female competition (Fig. 5.3c). Moreover, females were less than half as likely as were males to face intrasexual competition on a carcass (Fig. 5.3c). This suggests a novel direct benefit for female choice may be a reduction in the likelihood of female intrasexual competition.

Alternative Reproductive Tactics:

Kokko and Jennions (2008) note the importance of acknowledging alternative routes to fitness that may influence the nature of evolutionary responses. Because body size optima depend on context: larger males might maximize fitness (and circumvent the disadvantage of female preference for small males) by adopting a strategy that takes advantage of their superior competitive ability. This study reinforces the hypothesis presented in chapter 3, that males of different sizes may not experience the same competitive environment. This might lead to alternative male reproductive tactics with choice of tactic correlated with body size. For example, if large males have a competitive advantage at a carcass they might invest relatively more time searching for carrion than do small males (who may offset any disadvantage by calling females with whom to mate in the absence of a carcass). Beeler, Rauter & Moore (1999) inferred from a laboratory experiment that larger males of the congener *N. orbicollis* spend more time searching for carcasses while smaller males spend more time calling. Larger males, in their study, exhibited lower average frequency of pheromonal calling (without a carcass) than did smaller males, although proxies for searching behaviour did not differ between males of different size. If larger males of *N. vespilloides* were more successful than small males at locating

carcasses we would predict a large average size among males finding our unclaimed carcasses in experiment 2. In contrast, we found males that were first to arrive at carcasses (Fig. 5.3) tended to be smaller than the population mean body size. Thus, fitness benefits from carcass finding ability might be additive to success in attracting females to carcasses for small males. Moreover, if small males enjoy similar differential mating success when they employ the alternative mating tactic of calling females without a carcass, they stand to sire a disproportionate proportion of the offspring of females breeding alone.

Do large males actively seek calling males on a carcass?

The potential fitness of a larger than average male at a contested carcass is likely to be more resilient to the effects of direct competitors because he has more chance of securing dominant status and maximising his paternity (chapters 2 & 3). For the same reason, a larger male stands to benefit more than does a smaller male if he locates a carcass already claimed by other beetles, i.e., he may usurp former claimants. Even when a carcass is found to be occupied already by a male, but no female, a larger male could still benefit from the calling success of a smaller male. Müller and Eggert (1987) found that some males do arrive to calling males even in the absence of a carcass but we don't know whether these two groups of males differed in size. In experiment 2, because very few male beetles compared to female beetles were attracted to carcasses already claimed by males, the result is based on a small sample size in this group (n = 6) so evidence in support of this hypothesis remains equivocal.

Do males follow similar strategic rules regardless of body size with success depending on an interaction between the environment and body size?

Individual *N. vespilloides* may experience periods of low population density (spring emergence) and high density (overlapping generations in mid-late summer, Fig 1.3). Variation in frequency and intensity of competition may thus favour different phenotypes at different times of the year. This may be a factor in the evolution of reversible alternative reproductive tactics in this species. If being large is advantageous only in specific ecological contexts, i.e., when breeding resources are directly contested by same sex-rivals, the frequency of these contests becomes important. Thus a dynamic agent of selection acting on body size may be the shifting relationship between fluctuating population density and resource availability.

Conclusions:

We set out to test whether male investment between mating success and parental care was mediated by sex ratio cues which might provide information linking the likelihood of further mating opportunities and reproductive success. We found sex ratio experienced during post-eclosion development was not a significant determinant of behavioural strategy because the relationship between mating rate and reproductive success depends primarily on ecological context. Furthermore, this context is shaped by patterns of sexual selection which influence the social environment that breeding beetles encounter. Although smaller males seem to be at a disadvantage when mating at a carcass when there is male competition this is offset by their efficiency at attracting females. This has the result of reducing competition and potentially benefitting both caring partners. The prediction that large males have a general fitness advantage due to superior competitive ability was not

supported in nature. In this species, female mate choice, the social environment and alternative reproductive tactics lead to systems which can only be understood fully by including the influence of environmental factors. This study highlights the powerful and sometimes counterintuitive influence of ecological context. Similar experimental approaches in this system may offer the means to quantify total sexual selection alongside environmental agents of selection improving our understanding of the fundamental causes of adaptive evolution.

References:

Almada, V.C., Gonçalves, E.J., Oliveira, R.F. & Santos, A.J. (1995) Courting females:ecological constraints affect sex roles in a natural population of the blenniid fish *Salaria pavo*. *Animal Behaviour,* **49,** 1125-1127.

Andersson, M.B. (1994) *Sexual selection*. Princeton University Press, Princeton.

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

Beeler, A.E., Rauter, C.M. & Moore, A.J. (1999) Pheromonally mediated mate attraction by males of the burying beetle *Nicrophorus orbicollis*: alternative calling tactics conditional on both intrinsic and extrinsic factors. *Behavioral Ecology,* **10,** 578-584.

Beeler, A.E., Rauter, C.M. & Moore, A.J. (2002) Mate discrimination by females in the burying beetle Nicrophorus orbicollis: the influence of male size on attractiveness to females. *Ecological Entomology,* **27,** 1-6.

Clutton-Brock, T.H. & Huchard, E. (2013) Social competition and selection in males and females. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **368,** 20130074.

Clutton-Brock, T.H. & Vincent, A.C.J. (1991) Sexual selection and the potential reproductive rates of males and females. *Nature,* **351,** 58-60.

Creighton, J.C. (2005) Population density, body size, and phenotypic plasticity of brood size in a burying beetle. *Behavioral Ecology,* **16,** 1031-1036.

de Jong, K., Forsgren, E., Sandvik, H. & Amundsen, T. (2012) Measuring mating competition correctly: available evidence supports operational sex ratio theory. *Behavioral Ecology,* **23,** 1170-1177.

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

Eggert, A.K. & Müller, J.K. (1989) Mating success of pheromone-emitting Necrophorus males: do attracted females discriminate against resource owners? *Behaviour***,** 248-257.

Eggert, A.K. & Müller, J.K. (1997) Biparental care and social evolution in burying beetles: lessons from the larder. *The evolution of social behavior in insects and arachnids* (eds J.C. Choe & B.J. Crespi), pp. 216-236. Princeton University Press, Princeton.

Eggert, A.K. & Sakaluk, S.K. (1995) Female-coerced monogamy in burying beetles. *Behavioral Ecology and Sociobiology,* **37,** 147-153.

Emlen, S.T. & Oring, L.W. (1977) Ecology, sexual selection, and the evolution of mating systems. *Science,* **197,** 215-223.

Fromhage, L., McNamara, J.M. & Houston, A.I. (2007) Stability and value of male care for offspring: is it worth only half the trouble? *Biology letters,* **3,** 234-236.

Goldsmith, S.K. (1987) The mating system and alternative reproductive behaviors of *Dendrobias mandibularis* (Coleoptera: Cerambycidae). *Behavioral Ecology and Sociobiology,* **20,** 111-115.

Goldsmith, S.K. & Alcock, J. (1993) The mating chances of small males of the cerambycid beetle *Trachyderes mandibularis* differ in different environments (Coleoptera: Cerambycidae). *Journal of Insect Behavior,* **6,** 351-360.

Hopwood, P.E., Moore, A.J. & Royle, N.J. (2013) Nutrition during sexual maturation affects competitive ability but not reproductive productivity in burying beetles. *Functional Ecology,* **27,** 1350-1357.

House, C.M., Hunt, J. & Moore, A.J. (2007) Sperm competition, alternative mating tactics and context-dependent fertilization success in the burying beetle, *Nicrophorus vespilloides*. *Proceedings of the Royal Society B,* **274,** 1309-1315.

Hunt, J., Breuker, C.J., Sadowski, J.A. & Moore, A.J. (2009) Male-male competition, female mate choice and their interaction: determining total sexual selection. *Journal of Evolutionary Biology,* **22,** 13-26.

Kokko, H. & Jennions, M. (2008) Parental investment, sexual selection and sex ratios. *Journal of Evolutionary Biology,* **21,** 919-948.

Kokko, H. & Jennions, M. (2012) Sex differences in parental care. *The evolution of parental care* (eds N. Royle, P. Smiseth & M. Kölliker), pp. 101-116. Oxford University Press, Oxford.

Kokko, H., Klug, H. & Jennions, M.D. (2012) Unifying cornerstones of sexual selection: operational sex ratio, Bateman gradient and the scope for competitive investment. *Ecology Letters,* **15,** 1340-1351.

Kokko, H. & Rankin, D.J. (2006) Lonely hearts or sex in the city? Density-dependent effects in mating systems. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **361,** 319-334.

Kvarnemo, C. & Ahnesjo, I. (1996) The dynamics of operational sex ratios and competition for mates. *Trends in Ecology & Evolution,* **11,** 404-408.

Kvarnemo, C. & Ahnesjö, I. (2002) Operational sex ratios and mating competition. *Sex ratios: concepts and research methods* (ed. I.C.W. Hardy), pp. 366-382. Cambridge University Press, Cambridge.

Lee, V.E., Head, M.L., Carter, M.J. & Royle, N.J. (2013) Effects of age and experience on contest behaviour in the burying beetle, *Nicrophorus vespilloides*. *Behavioral Ecology,* **25,** 172-179.

MacColl, A.D.C. (2011) The ecological causes of evolution. *Trends in Ecology & Evolution,* **26,** 514-522.

Moore, A.J., Gowaty, P.A. & Moore, P.J. (2003) Females avoid manipulative males and live longer. *Journal of Evolutionary Biology,* **16,** 523-530.

Moore, A.J., Gowaty, P.A., Wallin, W.G. & Moore, P.J. (2001) Sexual conflict and the evolution of female mate choice and male social dominance. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* **268,** 517-523.

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

Müller, J.K. & Eggert, A.K. (1987) Effects of carrion-independent pheromone emission by male burying beetles (Silphidae: *Necrophorus*). *Ethology,* **76,** 297-304.

Müller, J.K. & Eggert, A.K. (1989) Paternity assurance by "helpful" males: adaptations to sperm competition in burying beetles. *Behavioral Ecology and Sociobiology,* **24,** 245-249.

Müller, J.K., Eggert, A.K. & Dressel, J. (1990) Intraspecific brood parasitism in the burying beetle, *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Animal Behaviour,* **40,** 491-499.

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

Pukowski, E. (1933) Ökologische untersuchungen an Necrophorus F. *Zoomorphology,* **27,** 518-586.

R Development Core Team (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org.

Shine, R. & Brown, G.P. (2008) Adapting to the unpredictable: reproductive biology of vertebrates in the Australian wet-dry tropics. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **363,** 363-373.

Steiger, S. (2013) Bigger mothers are better mothers: disentangling size-related prenatal and postnatal maternal effects. *Proceedings of the Royal Society B: Biological Sciences,* **280,** 20131225.

Steiger, S., Richter, K., Müller, J.K. & Eggert, A.K. (2007) Maternal nutritional condition and genetic differentiation affect brood size and offspring body size in Nicrophorus. *Zoology,* **110,** 360-368.

Trivers, R.L. (1972) *Parental investment and sexual selection*. Aldine, Chicago.

Wade, M.J. & Kalisz, S. (1990) The causes of natural selection. *Evolution,* **44,** 1947-1955.

Ward, R.J.S., Cotter, S.C. & Kilner, R.M. (2009) Current brood size and residual

reproductive value predict offspring desertion in the burying beetle

Nicrophorus vespilloides. *Behavioral Ecology*.

Chapter 6:

Individual resource specialisation versus intrasexual competitive success: the effect of body size in a heterogeneous environment.

Summary:

Being large can improve competitive ability and/or increase fecundity. However, fitness benefits related to size rely on opportunities for individuals to exploit their size advantage. For example, a female may only realise a potential fecundity advantage conferred by her relatively large size if available resources are sufficient to rear her maximum brood. Similarly, large, aggressive males could remain unrewarded if smaller male rivals can breed while avoiding direct male-male contests. Resource heterogeneity and variation in the frequency of contests over resources may therefore reduce potential reproductive benefits conferred by large size. The niche variation hypothesis predicts individual specialisation which might occur if individual resource-use is correlated with morphology. In burying beetles body size is hypothesised to covary positively with both a fecundity advantage (in females) and successful breeding resource defence (in females and males) but these advantages may be constrained by body size-related optima in breeding-resource size. We tested the prediction that 'bigger is better' in the wild, and in the laboratory, by varying breeding-resource size and quality related to the size of breeding burying beetle pairs. In the field, large beetles more often deserted small carcasses than large without breeding, but small beetles did not discriminate between carcass sizes. In the laboratory large beetle pairs reared more offspring on larger carcasses than did small beetle pairs, but the reverse was true on small carcasses. The results of both experiments suggest the relative value of a breeding resource differs according

to an individual's body size and support individual specialisation via body size in these beetles.

Key words: Individual specialisation; niche variation; body size; non-genetic inheritance; indirect genetic effect

Introduction:

Body-size is a fundamental attribute of an organism often correlated positively with measures of fitness (Brown, Marquet & Taper 1993; Blanckenhorn 2000; Roff 2002). Two general benefits of being large, with empirical support across a wide range of taxa, are increased fecundity in large females (Darwin 1874; Shine 1988; Roff 2002), and a competitive advantage for large individuals in contests for breeding resources or mating opportunities (Andersson 1994; Blanckenhorn 2005). Although limits exist to the benefits conferred by large body size (Schluter, Price & Rowe 1991) few studies have identified specific mechanisms opposing directional selection for increasing size and none have proposed selection for fecundity favouring small females (Blanckenhorn 2000).

There may be reproductive costs associated with increasing size; large females may suffer decreased fertility due to delayed reproduction and large males might suffer decreased mating success against smaller, more agile rivals. For example, Anholt (1991) manipulated larval density and food availability in the boreal bluet damselfly, *Enallagma boreale*, to produce adult phenotypes differing in size and time to emergence. Large males had higher survival to maturity but smaller males enjoyed greater mating success, perhaps due to lower flight costs enabling longer searches

for females. Similarly, Weissman *et al.* (2008) found that smaller males of an undescribed cricket (*Stenopelmatus* sp.) had a potential mating advantage over larger males due to a mechanical incompatibility when a male was larger than his mate.

Competition for scarce resources may favour larger individuals due to their relative success in contests for resources. Size differences among species may be maintained by stabilising selection acting on individuals that compete for similar resources in an ecological community (MacArthur & Levins 1967). There is evidence for this when several closely related species share the same range but segregate by size (or other phenotypic adaptations) according to the resources (e.g., habitat characteristics, prey size etc.) that they exploit (Schoener 1974; Werner & Gilliam 1984). However, Bolnick *et al*. (2003) argue that specialisation among individuals within species (apart from differences attributable to sexual dimorphism or ontogenetic progression) can also have profound effects on the evolutionary dynamics of a population. The niche variation hypothesis predicts that the niche width of a population can be wider than the niche width of an individual when intraspecific competition promotes individual resource-use specialisation (Van Valen 1965; Araújo, Bolnick & Layman 2011; Violle *et al.* 2012). This may occur when there are functional limitations of certain phenotypes (e.g., biomechanical trade-offs) preventing optimal exploitation of all available resource classes so phenotypic variation within a population itself may be adaptive (Wilson & Turelli 1986; Bolnick *et al.* 2003; Ackermann & Doebeli 2004; Bolnick *et al.* 2011). One prediction arising from the niche variation hypothesis is covariance between morphology and resource preference (Araújo, Bolnick & Layman 2011). However, a potential difficulty in testing

this prediction is defining an unambiguous axis of niche variation likely to be directly correlated with a morphological trait in a multidimensional ecological niche (Hutchinson 1957).

In burying beetles there is a clear axis of resource variation (carcass size) directly linked to variation in reproductive success. Burying beetles must use small vertebrate carcasses to reproduce, rearing a discrete brood in a single carcass. Because carcasses may be scarce, and intrasexual contests over these resources regularly occurs, previous studies on burying beetles have assumed a primary role for sexual selection in determining optimal body size predicting that increased competition should lead to stronger selection for larger size (e.g., Creighton 2005; Rauter *et al.* 2010). However there are ecological factors likely to influence selection on body size. For example, we might infer from sympatrically occurring congeneric burying beetles (that differ in body size) that the range of sizes of available carcasses provides a trophic niche whose fundamental width extends beyond the exploitative reach of a single species (Trumbo 1990a; Scott 1998). Correspondence between morphology and resource-use may be important in delimiting community structure and competitive interactions among species but the same pattern may exist within a species that has both wide variation in body size and available resources. One way that such specialisation might occur is if smaller beetles are more often usurped from more valuable, larger carcasses than are larger individuals. Alternatively, breeding beetles might preferentially utilize carcasses of a size appropriate to their own capabilities. For example, if maternal body size is related to fecundity large females will require a larger carcass than small females to rear an

optimal brood. These scenarios might therefore be interlinked because a small carcass may be relatively more valuable to a small beetle than to a large beetle.

We manipulated the relationship between carcass size (breeding resource) and body size of the burying beetle *Nicrophorus vespilloides* in the field and in the laboratory to look for evidence of individual specialisation related to morphology. We placed pairs of wild-caught beetles in two size categories (large or small) on mouse carcasses of different sizes (large or small) in the field and recorded frequency and identity and success of challengers. If relatively large size confers a benefit primarily via success in resource defence (i.e., sexual selection) we predicted that large beetles would be more successful in defending any breeding resource against usurpation by wild competitors. Alternatively, if the benefits conferred by body size are related to resource size leading to individual specialisation (i.e., niche variation) we predicted that successful resource defence would depend on beetle body size matching resource size reflecting differential relative reproductive value (e.g., smaller beetles may be more successful defending small carcasses because these offer a proportionally smaller marginal return for large beetles—who should have a lower threshold to decline in a contest and try elsewhere).

In the laboratory we measured the reproductive output of pairs of wild-caught beetles (in treatment categories the same as the wild experiment) to assess for differences in potential relative reproductive value related to interactions between resource size and body size. If the benefit of large body size is primarily the facilitation of competitive access to any carcass (i.e., regardless of its size), we predicted that with uncontested access to a carcass females should maintain offspring size over

offspring number and produce similar sized offspring (even at the expense of offspring number) across different sized carcasses. This is because larger offspring are expected to have greater resource holding potential as adults in the future. Moreover, reproductive output should either not differ between beetles of different size classes or larger beetles should have an advantage. Alternatively, support for niche variation would be context-specific reproductive output, e.g., favouring small beetles on small carcasses.

Methods:

Wild stock was captured using funnel-type bottle traps baited with putrescent beef or salmon during the summer of 2011 in mixed deciduous woodland (coord: N50° 11' 60", W5° 07' 05") for use in experiment 1, which was carried out in the field. Similar stock was obtained from the same woodland in 2012 for experiment 2. The capture site is approximately 1km from the experimental site (coord: N50° 11' 42", W5° 07' 51") and the two sites are separated by a main-road with open grass verges. Newly captured beetles were cleared of their phoretic mites (by blowing the mites off in the field—using a sharp exhalation of breath directed through pursed lips) and housed individually in the laboratory for a minimum of one week before being available for experiments. Beetles were fed *ad libitum* with decapitated mealworms, *Tenebrio molitor*, to ensure they were sexually mature and to reduce variation among individuals in their current nutritional status before their use in experiments.

Experiment 1 (field):

Captured wild beetles were assigned to size categories according to pronotal width and housed in the laboratory for between one and three weeks. Beetles with pronota

smaller or equal to 4.4mm were randomly allocated a partner within their same size category (i.e., male and female) to produce 'small' pairs, while beetles with pronota greater than or equal to 5.0mm were partnered one with another by the same process (these class size limits corresponded with the mean ±1 SD of a contemporaneous sample of 235 beetles from the wild population). The mean sizes of experimental beetles were: large female beetles: 5.29 ± 0.19mm (mean ± SD); small female beetles: 4.10 ± 0.18mm; large male beetles: 5.48 ± 0.20mm; small male beetles: 4.16 ± 0.25mm. Individuals assigned to pairs were kept singly until used in the experiment. All beetles were marked with a small dot of white office correction fluid applied to a pre-keyed area (using fine sandpaper) on either the scutellum or pronotum (randomized among pairs).

During July, August and September 2011, 77 Nicrocosms in total (as described in chapter 5) were distributed, no more than six at any one time, in 0.4 ha. of woodland with approximately 30m between each. Either a large or a small (thawed, pre-frozen) mouse carcass was placed inside each on natural soil substrate (no burial depth restriction was imposed on beetles). Small mice were 5.48 ± 0.28g (mean ± SD); large mice were 20.53 ± 0.31g. This size range is represented in nature by mature shrews, immature small mammals and songbird nestlings (~5g) through to adult small mammals and small passerines (~20g). It corresponds with other published studies on *N. vespilloides* that have shown use of carcasses between 2g, 3.5g and 5g to above 35g (e.g., Otronen 1988; Müller, Eggert & Furlkröger 1990; Smiseth & Moore 2002)

One pair of small or large beetles was introduced to each Nicrocosm (at approximately midday), containing a large or small carcass, before the natural late afternoon/early evening activity period of this beetle species. The experimental groups therefore consisted of a small beetle pair with either a large or a small carcass (SL or SS respectively); or a large beetle pair with either a large or a small carcass (LL or LS respectively).

Usually both introduced beetles burrowed into the substrate then emerged later in the afternoon to explore. Data from 64 Nicrocosms (out of a total of 77 trials) met our criterion for inclusion in analyses, which was that both experimental beetles of the pair made exploratory contact with the carcass during the later natural activity period of the same day in which they were released. A motion-sensitive infrared camera and infrared light emitting diodes in each Nicrocosm (see chapter 5) facilitated video data collection of information on number, species and gender of intruders, desertion and brood parasitic or satellite behaviour. Prenatal usurpation of the carcass was recorded and was defined as a challenge by a wild arriving conspecific or congeneric beetle(s) that successfully displaced a focal beetle from its dominant status on the carcass. Each reproductive event was terminated five days after carcass burial (or when larvae were first seen) and the occupants were disinterred to determine success in producing a clutch of eggs/larvae and confirm the number and identities of the adult beetles. These beetles were subsequently removed from the field site for the duration of the experiment to minimize any possible confounding artifact of their remaining and revisiting further experimental carcasses nearby. Empty Nicrocosms were cleaned and moved to a different location before fresh experimental occupants were introduced.

Experiment 2 (laboratory):

In the summer of 2012 wild-caught beetles were prepared and assigned to 80 pairs as for the experiment described above (two large females laid no eggs on one small and one large carcass respectively, and one small female laid infertile eggs on a large carcass. These three trials were re-established with new viable pairs to make a balanced n = 20 pairs in each group, total: n = 80). The factorial groups were the same as for experiment 1 and the experimental design was similar except that beetle pairs bred under controlled conditions in the laboratory. Large female beetles were 5.32 ± 0.26mm (mean ± SD); small female beetles were 3.97 ± 0.17mm. Large male beetles were 5.45 ± 0.24mm; small male beetles were 4.08 ± 0.23mm. Each experimental pair was provided with a mouse carcass (small = 5.54 ± 0.28g (mean ± SD); large = 20.54 ± 0.31g) in a plastic breeding box (17 × 11 × 5cm) with about 3cm moist compost to enable natural reproductive behaviour. Beetles were undisturbed for the duration of each reproductive bout and larvae produced were weighed and measured at dispersal i.e., when fully developed with at least two witnessed wandering from the depleted carcass remains (Rauter & Moore 2002).

Statistics:

Experiment 1 (field):

Rejection of breeding resources was specified as both partners making exploratory contact with the carcass (see chapter 4) but voluntarily leaving (i.e., without being forcibly evicted by intruders) within four days and with no evidence of eggs or larvae on disinterment. Rejection was analysed using binary logistic regression with treatment as a single fixed factor with four levels (the four combinations of carcass

and beetle pair sizes). Rejection was also analysed, using the same criteria, for female behaviour alone (i.e., regardless of whether males stayed to call after a female had rejected a carcass and left). Burial duration is defined as the number of hours (natural log to normalise distribution) from either partner's first exploratory contact until the moment the whole carcass is covered from view to the nearest hour. The analysis was a linear model with treatment (i.e., the four treatment combinations) as the single independent variable. Only carcasses buried by one or both of the experimental beetles were included in this analysis. Usurpation of the breeding resource was defined as either one (or both although this did not occur) of the focal pair being displaced by a wild beetle before any larvae arrived (wild beetles never arrived after the carcass was buried in this study). Usurpation was analysed using a general linear model with binomial errors and beetle size and carcass size as fixed factors. 'R' version 2.14.1 (R Development Core Team 2011) was used for all analyses and models were simplified using a stepwise approach (Crawley 2007).

Experiment 2 (laboratory):

Larval number had a bimodal distribution and each carcass size was analysed separately with Welch's two sample t-tests. Mean offspring size (natural log transformed to normalise distribution) was analysed using a linear model with carcass size (large or small) and parental pair size (large or small) as categorical fixed factors. All analyses were performed using 'R' version 2.14.1 (R Development Core Team 2011) and means are presented with standard errors throughout.

Results:

Experiment 1 (field):

Treatment (the combination of carcass and beetle size categories) had a significant effect on breeding resource utilisation (GLM, treatment: χ^2_3 = 18.332, p < 0.001). Large beetle pairs voluntarily deserted a greater proportion of small than large carcasses without breeding (GLM, carcass size: χ^2_1 = 8.362, p = 0.004). Small beetle pairs did not differ in the proportion of large or small carcasses utilised (GLM, carcass size: χ^2_1 = 1.419, p = 0.234). The desertion of carcasses (i.e., without utilising them for breeding) by large beetle pairs was robust to analysis using female desertion alone as the response (GLM, carcass size: χ^2_1 = 7.197, p = 0.007, Fig. 6.1a) because small carcasses did not remain viable for long enough (due to slug scavengers) to allow a deserted male partner time to call a new mate. Duration of carcass burial by focal beetles (excluding rejected carcasses) differed among treatments with small beetles burying small carcasses more rapidly than did large beetles or than large carcasses were buried by beetles of any size (LM, treatment: $F_{3,45}$ = 3.786, p = 0.017, Fig. 6.1b).

The incidence of usurpation by intruders was low: 14 out of 64 carcasses were visited by wild conspecific intruders. Out of 54 successful broods six had one partner displaced forcibly by a rival (two focal males: one large male usurped from large carcass and one small male on a small carcass; and four focal females: one large female from a large carcass; two small females from large carcasses and one small female from a small carcass). One further female (small on small carcass) was replaced by an intruding beetle that did not breed successfully. Neither beetle size (GLM, χ^2_1 = 1.486, p = 0.223), carcass size (χ^2_1 = 0.161, p = 0.688) nor the interaction between them (GLM, χ^2_1 = 2.98, p = 0.084) predicted usurpation of the

breeding resource. There was no pattern supporting an explanatory trend in this interaction.
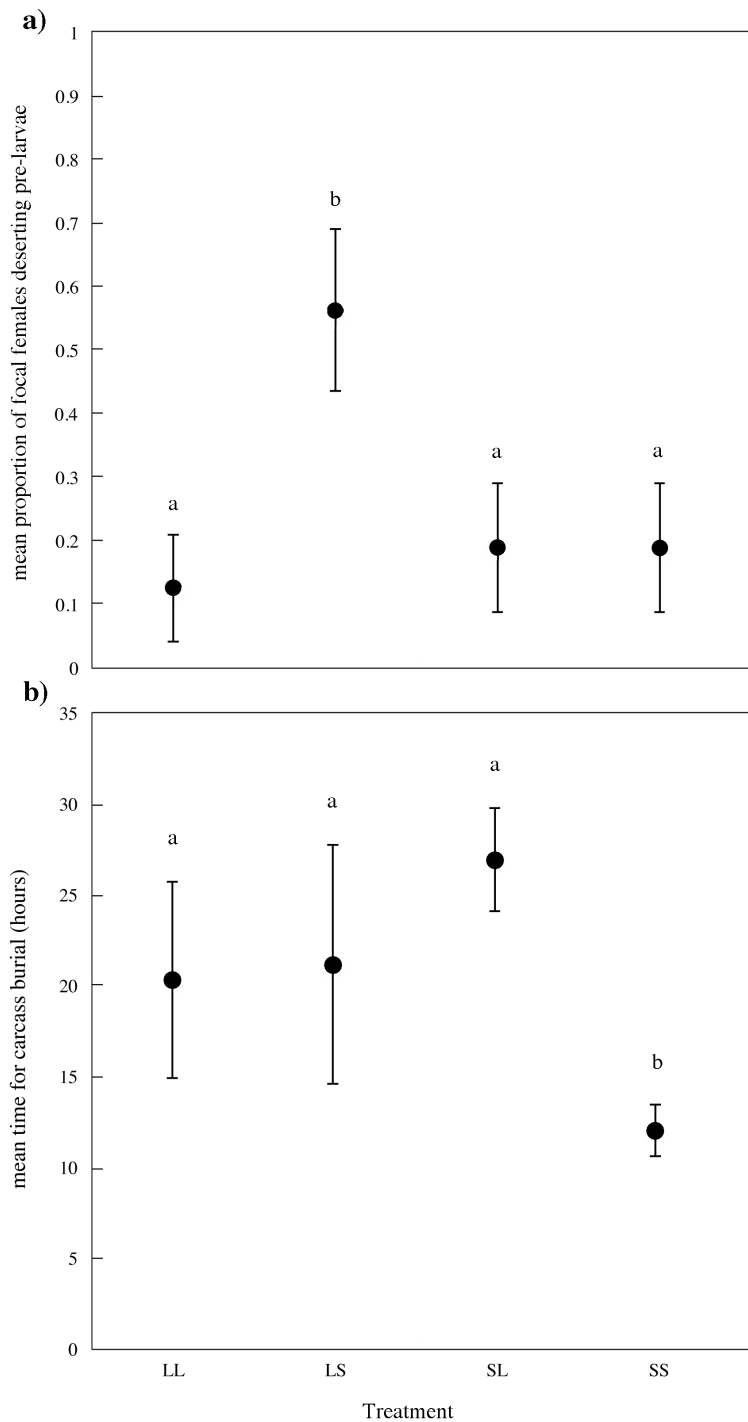
Figure 6.1: a) In the wild, large female beetles were less likely to use a small carcass for breeding than were small female beetles. There was no difference between female beetles of different size in utilizing large carcasses. b) Duration of carcass interment by focal beetles successfully rearing larvae. Treatment categories: LL = large beetle pair & large carcass; LS = large beetle pair & small carcass; SL = small beetle pair & large carcass; SS = small beetle pair & small carcass.

Experiment 2 (laboratory):

Large beetle pairs were more productive than small beetles on large carcasses; they reared broods containing more offspring than did small beetles (Welch's unpaired t. test, parental pair size: t = 2.753, est. *d.f.* = 34.287, p < 0.01, Fig. 6.2a). However the reverse was true when beetles were provided with a small carcass: small beetles reared more offspring on average than large beetles (parental pair size: t = 2.168, est. *d.f.* = 37.72, p < 0.05, Fig. 6.2a).

When controlling for carcass size (log) mean individual offspring mass at dispersal did not differ between large or small parental pairs (LM, parental pair size; $F_{1,77}$ = 0.373, p = 0.543, Fig. 6.2b). However, mean individual offspring mass was affected by carcass size; large carcasses produced offspring of greater mean size (carcass size; $F_{1,78}$ = 77.108, p < 0.001, Fig. 6.2b).
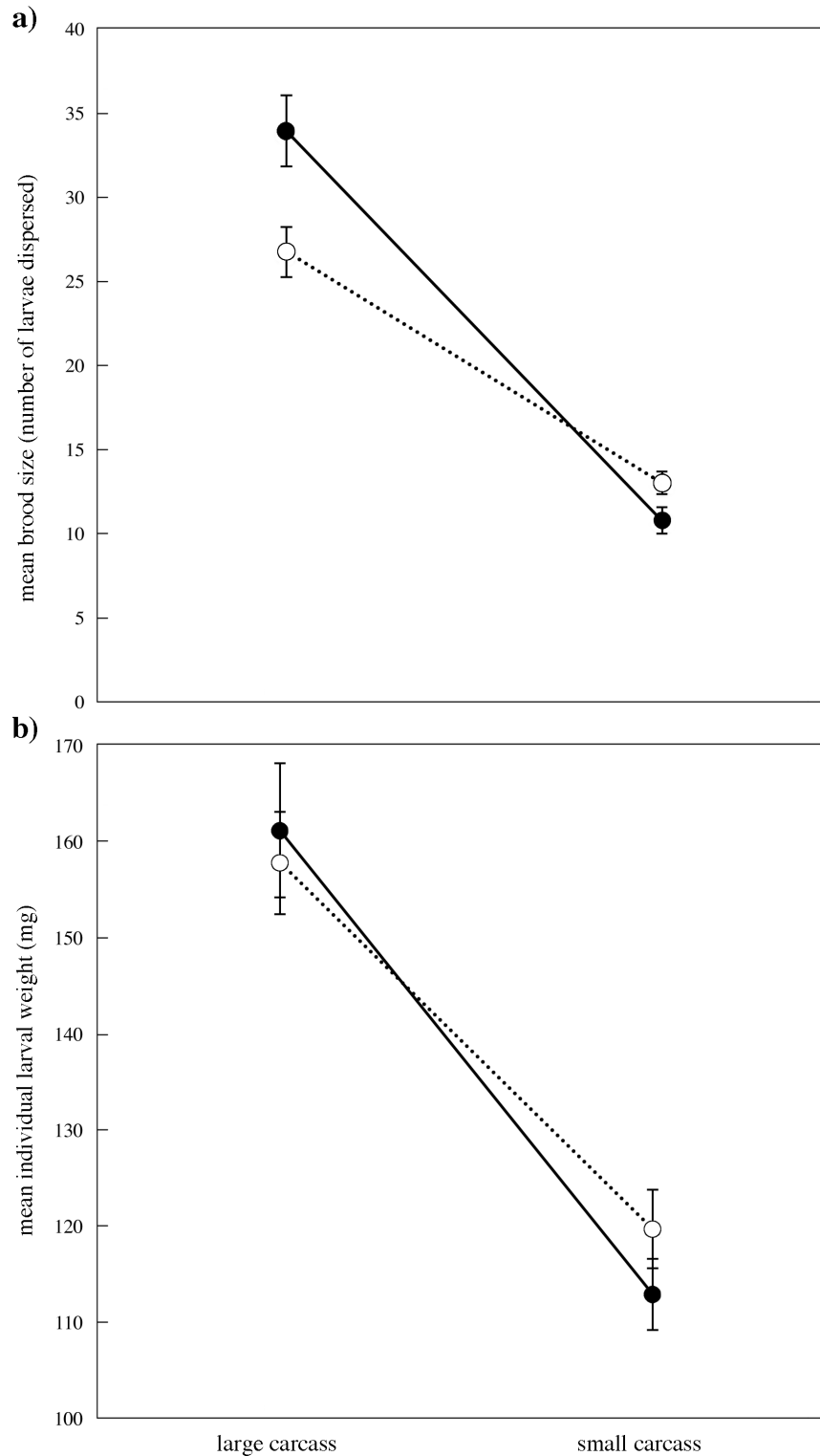
Figure 6.2: a) Large beetles successfully rear more larvae than small beetles on large carcasses, but the reverse is true for small beetles on small carcasses. b) Individual larval mean weight is smaller when broods are reared on small carcasses, independent of parental size. Solid circles = large pairs; open circles = small pairs.

Discussion:

We found evidence of individual specialisation in resource use in *N. vespilloides* in the field. Large beetles were significantly more likely to reject small carcasses than large carcasses. Small beetles did not differentiate between carcasses of the two size categories (Fig. 6.1a). This was not due to any intrinsic incompatibility due to large body size (e.g., large beetles might require a large proportion of carrion from a small carcass for their own nutrition rendering successful breeding impossible); in controlled laboratory conditions similarly matched large beetle pairs raised broods with a mean of 10.7 larvae on carcasses of similar size as those provided in the field (Fig. 6.2a). Further evidence of an assortative preference between beetle size and carcass size comes from the speed that beetles interred carcasses that were utilised successfully for breeding. Small beetles buried small carcasses more rapidly than large beetles did (Fig. 6.1b). Small beetles also took on average longer than 24 hours to cover large carcasses (Fig. 6.1b), this potentially is a critical time threshold beyond which unburied resources remain at risk of detection during the following day's peak beetle and slug activity period.

In support of our previous findings (chapter 5), once a female beetle, or pair of beetles had possession of a carcass in the wild they were more likely than not to retain it. There is considerable evidence that even a slight relative size advantage predicts success in intraspecific contests in these beetles (Bartlett & Ashworth 1988; Otronen 1988; Hopwood, Moore & Royle 2013; Lee *et al.* 2013) and may aid successful resource defence against congeneric beetle competitors (Robertson 1993; Trumbo 2006). However, the extent of any disadvantage for small individuals depends on the frequency and impact of such competitive encounters, and/or takeover attempts, in an ecological context. Here we found that less than 24% of

total carcasses (i.e., 15 out of 64 carcasses) attracted intruding conspecific rivals and of those that did only 6 intruding individuals successfully usurped one or other of the current resource holding beetles in a successful breeding event (out of 54 carcasses that produced larvae). No carcasses were lost to congeneric competitors. This low incidence of usurpation events limits statistical power to detect an effect of differential body size on contest success. Relative body size is important in the context of direct conspecific contests for resources, and in predicting successful resource defence against congeneric beetle competitors in some species of burying beetles (Robertson 1993; Trumbo 2006). However, any overall disadvantage for small individuals remains hypothetical unless the frequency and impact of such encounters is known in an ecological context.

Body size, contest success and population density:

In burying beetles parents compensate for size variation among carcasses by matching offspring number to resource quantity (Eggert & Müller 1997; Scott 1998). In some species this correction is sufficiently accurate that mean offspring size is similar across a range of carcass sizes (e.g., *N. tormentosus*, Trumbo 1990b; *N. orbicollis*, Trumbo & Fernandez 1995a). Eggert and Müller (1997) propose that the optimum compromise between size and number of offspring ought to be independent of carcass size, reflecting instead the intraspecific competitive environment likely to confront offspring when they themselves reproduce. Creighton (2005) interpreted a positive correlation between inferred population density of *N. orbicollis* in different geographic locations and body size, as evidence of phenotypic plasticity in parental brood thinning behaviour. His interpretation was that with increasing population density frequency of contests for carcasses increases and the benefit conferred by

size in contests tips the optimum trade-off between offspring size and number towards large size. Beetle parents are hypothesised to gain an indirect benefit, through greater offspring contest success, when they respond to cues indicating high population density—and then facultatively reduce brood sizes, relative to carcass sizes (thereby producing fewer but larger offspring) when there appears currently to be higher probability of encountering rivals at a carcass. Under this hypothesis sexual selection may influence plasticity in brood tailoring behaviour in a complementary way in both sexes.

Steiger *et al.* (2007) challenged this interpretation, finding genetic differences in brood thinning behaviour between populations of *N. defodiens* that resulted in different sized offspring and also (using *N. vespilloides*) showed that food stressed females were constrained to produce smaller broods with larvae that grow larger (on control carcasses) as a consequence. Rauter *et al.* (2010) manipulated cues indicating contest frequency in *N. pustulatus* but found no evidence in support of adaptive reproductive behaviour in accord with a shifting predicted optimal trade-off between offspring body size and number.

Both Creighton (2005) and Rauter *et al.* (2010) used predictions generated by the formalised game-theoretic model developed by Mesterton-Gibbons & Hardy (2004) that increased frequency of body-size-dependent contests should select for larger body size via decreasing clutch-size optima. This theory has empirical support (e.g., in a parasitoid wasp: Goubault, Mack & Hardy 2007) but there are reasons why the predictions might break down in burying beetles. Mesterton-Gibbons & Hardy (2004) provide three assumptions under which their model may be generalized (i.e., smaller

broods produce larger individuals; breeding resources are scarce; contest success for resources is body size related). However, our study joins others in showing that the relationship between brood size and offspring size depends on carcass size (e.g., Bartlett & Ashworth 1988; chapter 3; Eggert & Müller 1997). Furthermore, studies of burying beetles in nature have often found evidence that a majority of successful reproductive events are uncontested, i.e., monogamous pair or uniparental female (e.g., Müller, Eggert & Dressel 1990; Eggert 1992; chapter 5) but see (Müller *et al.* 2007). Investigating body size variation in burying beetles therefore ought not to be predicated on the assumption that contest ability related to body size is sufficient to select for increased size. The low incidence of carcass usurpation by conspecific competitors in this and our other field experiments (see chapter 5) with *N. vespilloides* suggests that, at least in our study population, contest success via a body size advantage may not be the primary determinant of successful reproduction. A non-mutually exclusive alternative explanation for among population morphological variation is individual niche variation affecting the distribution of a correlated phenotypic trait. For example, in a recent study Hsu *et al.* (2014) reported increases in trophic niche width associated with greater variation in bill morphology among populations of the passerine *Paradoxornis webbianus*. In burying beetles a similar effect might be produced when carcass size influences body size: the distribution of body size of resident beetles will likely covary among populations experiencing different local communities of vertebrates.

Body size, resource variation and community structure:

Carcass availability imposes fundamental limits on burying beetle reproduction (and therefore population density). *N. vespilloides* is the smallest of five congeneric

burying beetles in our study population (*N. humator, N. investigator, N. interruptus, N. vespillo and N. vespilloides* in descending order of mean body size). There is documented character displacement among species along several axes including body size and seasonal activity period that appears to permit coexistence in communities of these beetles which share similar life-history, morphology and guild (Pukowski 1933; Scott 1998). Comparable burying beetle communities are also described in other countries with different constituent species (e.g., Müller & Eggert 1987; Otronen 1988; Scott & Traniello 1990; Trumbo 1994; Suzuki 2000). As the smallest species in Europe *N. vespilloides* is unlikely to compete with congenerics for very small carcasses. However, here we present evidence supporting niche complementarity: individual variation that may aid in reducing intraspecific competition (Bolnick *et al.* 2011). Within the large range of body sizes of *N. vespilloides* (general introduction Fig. 6.1a) we found individual sensitivity to the size of breeding resource that supports the niche variation hypothesis: larger individuals were less likely to utilize small carcasses for breeding. Assuming that carcasses vary in size any potential fecundity advantage held by large females would be weakened if they accepted breeding resources of insufficient size to maximize fecundity because searching for alternatives might be more profitable.

Mechanisms of selection on body size:
In an experimental study of parental performance in the congeneric *N. pustulatus* Rauter and Moore (2002) partitioned offspring phenotypic variance into direct and indirect genetic components using standardised, cross-fostered broods. Their results suggest that indirect genetic effects of parental care in these beetles may have a significant influence on the response of offspring growth and development to

selection on body mass and size. Their experimental design assumed no indirect environmental effects (but nonetheless exposed an environmental effect of carcass size). Parental care may mediate offspring growth and development via feeding and carcass maintenance, but in burying beetles, as well as feeding offspring and providing a suitable rearing environment, there is an important additional metric of parental care performance. This is actively matching the number of offspring to the size of the breeding carcass and can be viewed as an interaction between the direct environmental effect of variation in carcass size and indirect genetic effects of maternal control (or physiological limitation) of oviposition and parental infanticidal behaviour, i.e., the rules that parents use to tailor offspring number to carcass size. These rules are unknown beyond evidence that carcass volume appears to be the cue indicating resource size (Trumbo & Fernandez 1995b) and might differ between males and females (e.g., perhaps based on prior information about oviposition in females). However, there is a strong environmental component determining body size variation in *N. vespilloides* with studies (including this study) reporting a positive correlation between carcass size and individual offspring (and therefore adult) size (Bartlett & Ashworth 1988; Eggert & Müller 1997; chapter 3) a finding also reported in the similarly small *N. defodiens* (Scott & Traniello 1990).

There are other aspects of body size related to fitness in this species that may help to explain this pattern. We previously found that smaller males avoid contests by preferentially attracting females to carcasses potentially undermining the relationship between contest ability and reproductive success. Reproductive success also depends on differential success in locating carcasses (chapter 5) and the social environment encountered at a potential breeding carcass. In the laboratory we

controlled for these factors and results support a prediction in concordance with the niche variation hypothesis that functional trade-offs may lead to individual specialisation positively correlated with morphological variation (Wilson & Turelli 1986; Bolnick *et al.* 2003). We found an apparent trade-off directly involving reproductive potential because large beetles reared over 20% more offspring on large carcasses than did small beetles but were outperformed themselves by small beetles on small carcasses. It is possible that small females were simply unable to produce eggs enough to populate large carcasses. However, there is a puzzle because small beetles having produced fewer offspring on a larger carcass nevertheless reared individuals of similar size as did large beetles. The expectation would be for larger offspring to be produced when fewer compete for the same finite resource. Larvae did not achieve their physiological maximum body size in either carcass treatment as larvae of this species grow to over 200mg when the relationship between brood size and carcass size is manipulated experimentally, even on intermediate carcass sizes (e.g., Lock *et al.* 2007). The same pattern but reversed (i.e., offspring mean mass reflected carcass mass, not offspring number or parental body size) was observed on small carcasses suggesting that costs of the provision of parental care related to size were unlikely to account for the effect.

Bet-hedging or coin-flipping?

Because the brood tailoring rules of parenting behaviour provide a means to alter the relationship between carcass size and offspring size relatively simple rules may produce a form of diversified bet-hedging analogous to the adaptive 'coin-flipping' principle of Cooper and Kaplan (1982) where ontogenic random variation among offspring is adaptive in an unpredictable environment. In this instance the outcome of

the coin-flip (the size of offspring) is influenced by an environmental component affecting offspring development: the unpredictable size of the breeding resource. There might be fitness benefits arising from parental behavioural rules of thumb that result in such a marked environmental influence on offspring size. For example, parents might gain indirect benefits if mean offspring size tracks seasonal changes in size distribution of available carcasses (e.g., a spring glut of failed fledglings or abandoned small mammal neonates). Small offspring (from a small carcass) may find an environment in which they are favoured by disproportionate availability of smaller carcasses (rejected or under-utilised by larger beetles), and should they locate a large carcass themselves they have potential to produce progeny larger than themselves (Fig. 6.2b) . Our laboratory findings suggest that there is no single optimal body size for *N. vespilloides* given environmental heterogeneity (in breeding resource size). In this case a significant dynamic agent of selection on body size may be environmental heterogeneity in carcass size mediated by parental brood tailoring behaviour. This potentially powerful component of parental performance (the indirect genetic effect of variation in parental brood tailoring rules) needs further investigation.

Non-genetic effects?

Fox & Mousseau (1998) hypothesised that a transgenerational non-genetic phenotypic correlation could be maintained in a randomly mating population if female early host experience (e.g., in a herbivorous insect) influenced both oviposition preference and offspring performance. This is distinct from an 'indirect' genetic effect that is genetically based in respect of heritability of a parental phenotype that influences offspring phenotypes. In our laboratory experiment we found that offspring

body size reflected differences in carcass size independent of parental size variation (Fig. 6.2b). Assortative carcass use observed in the field therefore provides a novel case of a non-genetic mechanism of phenotypic inheritance that might produce stable transgenerational patterns of differences in size and behaviour. This may occur because by rejecting small carcass in favour of large for breeding, large mothers are 'reconstructing' the developmental environment that influenced their own body size (Uller 2012; Uller & Helanterá 2013).

Conclusions:

Individual contest success in burying beetles has previously been shown to be dependent on body size differences between competitors. However, we found no evidence that pairs of small beetles *per se* were at greater risk of losing breeding resources than were pairs of large beetles in the field. We found evidence that a significant proportion of large beetles (but not small) rejected small potential breeding resources in the field. In laboratory conditions where opportunities for deserting were restricted this did not occur and pairs of beetles reproduced successfully regardless of their size or the size of the carcass. Maximum reproductive output depended on an optimal match between resource size and parental body size supporting an interpretation of within species niche complementarity. Individual preference for breeding resource use depended on the relationship between resource size and body size and body size itself has a significant environmental component being determined by the carcass size on which the individual was reared. This suggests an environmental mechanism by which transgenerational inheritance of body size might occur in this species.

References:

Ackermann, M. & Doebeli, M. (2004) Evolution of niche width and adaptive diversification. *Evolution,* **58,** 2599-2612.

Andersson, M.B. (1994) *Sexual selection*. Princeton University Press, Princeton.

Anholt, B.R. (1991) Measuring selection on a population of damselflies with a manipulated phenotype. *Evolution,* **45,** 1091-1106.

Araújo, M.S., Bolnick, D.I. & Layman, C.A. (2011) The ecological causes of individual specialisation. *Ecology Letters,* **14,** 948-958.

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

Blanckenhorn, W.U. (2000) The evolution of body size: what keeps organisms small? *Quarterly Review of Biology,* **75,** 385-407.

Blanckenhorn, W.U. (2005) Behavioral causes and consequences of sexual size dimorphism. *Ethology,* **111,** 977-1016.

Bolnick, D.I., Amarasekare, P., Araújo, M.S., Bürger, R., Levine, J.M., Novak, M., Rudolf, V.H.W., Schreiber, S.J., Urban, M.C. & Vasseur, D.A. (2011) Why intraspecific trait variation matters in community ecology. *Trends in Ecology & Evolution,* **26,** 183-192.

Bolnick, D.I., Svanbäck, R., Fordyce, J.A., Yang, L.H., Davis, J.M., Hulsey, C.D. & Forister, M.L. (2003) The ecology of individuals: incidence and implications of individual specialization. *The American Naturalist,* **161,** 1-28.

Brown, J.H., Marquet, P.A. & Taper, M.L. (1993) Evolution of body size: consequences of an energetic definition of fitness. *American Naturalist,* **142,** 573-584.

Cooper, W.S. & Kaplan, R.H. (1982) Adaptive 'coin-flipping': a decision-theoretic examination of natural selection for random individual variation. *Journal of Theoretical Biology,* **94,** 135-151.

Crawley, M.J. (2007) *The R book*. Wiley and Sons, Chichester.

Creighton, J.C. (2005) Population density, body size, and phenotypic plasticity of brood size in a burying beetle. *Behavioral Ecology,* **16,** 1031-1036.

Darwin, C. (1874) *The descent of man, and selection in relation to sex,* 2nd edn. John Murray, London.

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

Eggert, A.K. & Müller, J.K. (1997) Biparental care and social evolution in burying beetles: lessons from the larder. *The evolution of social behavior in insects and arachnids* (eds J.C. Choe & B.J. Crespi), pp. 216-236. Princeton University Press, Princeton.

Fox, C.W. & Mousseau, T.A. (1998) Maternal effects as adaptations for transgenerational phenotypic plasticity in insects. *Maternal effects as adaptations* (eds T.A. Mousseau & C.W. Fox), pp. 159-177. Oxford University Press, Oxford.

Goubault, M., Mack, A.F.S. & Hardy, I.C.W. (2007) Encountering competitors reduces clutch size and increases offspring size in a parasitoid with female-female fighting. *Proceedings of the Royal Society B: Biological Sciences,* **274,** 2571-2577.

Hopwood, P.E., Moore, A.J. & Royle, N.J. (2013) Nutrition during sexual maturation affects competitive ability but not reproductive productivity in burying beetles. *Functional Ecology,* **27,** 1350-1357.

Hsu, Y.-C., Shaner, P.-J., Chang, C.-I., Ke, L. & Kao, S.-J. (2014) Trophic niche width increases with bill-size variation in a generalist passerine: a test of niche variation hypothesis. *Journal of Animal Ecology,* **83,** 450-459.

Hutchinson, G.E. (1957) Cold Spring Harbor Symposium on Quantitative Biology. *Concluding remarks,* **22,** 415-427.

Lee, V.E., Head, M.L., Carter, M.J. & Royle, N.J. (2013) Effects of age and experience on contest behaviour in the burying beetle, *Nicrophorus vespilloides*. *Behavioral Ecology,* **25,** 172-179.

Lock, J.E., Smiseth, P.T., Moore, P.J. & Moore, A.J. (2007) Coadaptation of prenatal and postnatal maternal effects. *American Naturalist,* **170,** 709-718.

MacArthur, R. & Levins, R. (1967) The limiting similarity, convergence, and divergence of coexisting species. *The American Naturalist,* **101,** 377-385.

Mesterton-Gibbons, M. & Hardy, I.C.W. (2004) The influence of contests on optimal clutch size: a game-theoretic model. *Proceedings of the Royal Society of London, Series B: Biological Sciences,* **271,** 971-978.

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

Müller, J.K. & Eggert, A.K. (1987) Effects of carrion-independent pheromone emission by male burying beetles (Silphidae: *Necrophorus*). *Ethology,* **76,** 297-304.

Müller, J.K., Eggert, A.K. & Dressel, J. (1990) Intraspecific brood parasitism in the burying beetle, *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Animal Behaviour,* **40,** 491-499.

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

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

Pukowski, E. (1933) Ökologische untersuchungen an Necrophorus F. *Zoomorphology,* **27,** 518-586.

R Development Core Team (2011) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org.

Rauter, C.M., Mcguire, M.J., Gwartney, M.M. & Space, J.E. (2010) Effect of population density and female body size on number and size of offspring in a species with size-dependent contests over resources. *Ethology,* **116,** 120-128.

Rauter, C.M. & Moore, A.J. (2002) Evolutionary importance of parental care performance, food resources, and direct and indirect genetic effects in a burying beetle. *Journal of Evolutionary Biology,* **15,** 407-417.

Robertson, I.C. (1993) Nest intrusions, infanticide, and parental care in the burying beetle, *Nicrophorus orbicollis* (Coleoptera: Silphidae). *Journal of Zoology,* **231,** 583-593.

Roff, D. (2002) *Life History Evolution*. Sinauer Associates, Massachusetts.

Schluter, D., Price, T.D. & Rowe, L. (1991) Conflicting selection pressures and life history trade-offs. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* **246,** 11-17.

Schoener, T.W. (1974) Resource partitioning in ecological communities. *Science,* **185,** 27-39.

Scott, M.P. (1998) The ecology and behavior of burying beetles. *Annual Review of Entomology,* **43,** 595-618.

Scott, M.P. & Traniello, J.F.A. (1990) Behavioural and ecological correlates of male and female parental care and reproductive success in burying beetles (Nicrophorus spp.). *Animal Behaviour,* **39,** 274-283.

Shine, R. (1988) The evolution of large body size in females: a critique of Darwin's" fecundity advantage" model. *The American Naturalist,* **131,** 124-131.

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

Steiger, S., Richter, K., Müller, J.K. & Eggert, A.K. (2007) Maternal nutritional condition and genetic differentiation affect brood size and offspring body size in Nicrophorus. *Zoology,* **110,** 360-368.

Suzuki, S. (2000) Changing dominantñsubordinate relationships during carcass preparation between burying beetle species (Nicrophorus: Silphidae: Coleoptera). *Journal of Ethology,* **18,** 25-28.

Trumbo, S. & Fernandez, A. (1995a) Regulation of brood size by male parents and cues employed to assess resource size by burying beetles. *Ethology Ecology & Evolution,* **7,** 313-322.

Trumbo, S.T. (1990a) Interference competition among burying beetles (Silphidae, Nicrophorus). *Ecological Entomology,* **15,** 347-355.

Trumbo, S.T. (1990b) Regulation of brood size in a burying beetle, Nicrophorus tomentosus (Silphidae). *Journal of Insect Behavior,* **3,** 491-500.

Trumbo, S.T. (1994) Interspecific competition, brood parasitism, and the evolution of biparental cooperation in burying beetles. *Oikos,* **69,** 241-249.

Trumbo, S.T. (2006) Infanticide, sexual selection and task specialization in a biparental burying beetle. *Animal Behaviour,* **72,** 1159-1167.

Trumbo, S.T. & Fernandez, A.G. (1995b) Regulation of brood size by male parents and cues employed to assess resource size by burying beetles. *Ethology Ecology & Evolution,* **7,** 313-322.

Uller, T. (2012) Parental effects in development and evolution. *The evolution of parental care* (eds N.J. Royle, P.T. Smiseth & M. Kölliker), pp. 247-266. Oxford University Press, Oxford.

Uller, T. & Helanterá, H. (2013) Non-genetic inheritance in evolutionary theory: a primer. *Non-Genetic Inheritance,* **1,** 27-32.

Van Valen, L. (1965) Morphological variation and width of ecological niche. *American Naturalist,* **99,** 377-390.

Violle, C., Enquist, B.J., McGill, B.J., Jiang, L., Albert, C.H., Hulshof, C., Jung, V. & Messier, J. (2012) The return of the variance: intraspecific variability in community ecology. *Trends in Ecology & Evolution,* **27,** 244-252.

Weissman, D.B., Judge, K.A., Williams, S.C., Whitman, D.W. & Lee, V.F. (2008) Small-male mating advantage in a species of Jerusalem cricket (Orthoptera: Stenopelmatinae: *Stenopelmatus*). *Journal of Orthoptera Research,* **17,** 321-332.

Werner, E.E. & Gilliam, J.F. (1984) The ontogenetic niche and species interactions in size-structured populations. *Annual Review of Ecology and Systematics,* **15,** 393-425.

Wilson, D.S. & Turelli, M. (1986) Stable underdominance and the evolutionary

invasion of empty niches. *American Naturalist,* **127,** 835-850.

Chapter 7:

General discussion

The aim of this thesis was to examine the effects of variation in the level and predictability of the nutritional and social environment experienced by individuals on the expression of parental and offspring traits. Because the structure of this thesis takes the form of discrete research manuscripts this discussion will present a synthesis and précis of each chapter noting novel and incremental findings where they occur but also making connections between the findings to put them into a wider context.

Chapter 2: **Nutrition during sexual maturation affects competitive ability but not reproductive productivity in burying beetles.**

In this chapter, availability of nutrition post-eclosion was manipulated to simulate an adult but reproductively immature beetle experiencing a delay finding the necessary resources to complete sexual development. Treated beetles survived the period of starvation and their parental performance (in terms of surrogate and own offspring successfully reared over three reproductive bouts) and longevity did not differ from controls. However, treated beetles (delayed feeding) suffered reduced contest success (independent of body size).

The conclusions from this chapter are that parents allocate resources preferentially towards reproductive function maintaining parental performance and offspring fitness at the expense of contest success. Differential size of direct competitors largely overwhelms other sources of individual variation shifting the importance of the probability of contest success towards an ecological question about the frequency that these contests take place (or rather, the proportion of breeding bouts in which

the carcasses are contested). This is because unless parents can secure a carcass they will never get the opportunity to exercise their preserved reproductive capacity. These data add incrementally to evidence that the timing of nutritional variation may be important particularly if variation coincides with a key developmental window.

Chapter 3: Effects of resource variation during early life and adult social environment on contest outcomes in burying beetles: silver spoon or context-dependent strategy?

Beetles can maintain their parental performance (fecundity) even when competitive ability is compromised by delayed nutrition during development (chapter 2). However, in order to become parents beetles may have to fight against rival same-sex opponents for access to breeding resources (a carcass). In this chapter I investigated potential success in a variable social environment with the quality of the adult social environment defined by the relative size of focal beetles compared to that of their opponent. Because the nutritional environment was manipulated at two important developmental life-cycle stages (i.e., larval growth and development and post-eclosion reproductive maturation) I was able to test alternative scenarios relating to the effects of the early developmental environment on success later in life. A novel approach for this type of study was including a measure of adult environmental quality against which the effects of the differing early environments on the competitive success of individuals could be measured. Many studies have found, as did I, support for silver spoon effects. However, a lack of unambiguous empirical support for environmental-matching in the literature as well as in this study might reflect non-generalisable features of the human life-cycle from which the hypothesis was developed (Hales & Barker 1992). In humans, socio-economic and cultural

factors may strengthen the correlation between the environment experienced through a prolonged development and that encountered in adulthood, reinforcing the predictive power of early conditions (Victora *et al.* 2008). In contrast, some species do not feed at all as adults (e.g., mayflies, Ephemeroptera; crane flies, Tipulidae; glow worms, e.g., *Lampyris noctiluca* and even some vertebrates, e.g., American brook lamprey, *Lethenteron appendix*).

Because I manipulated the larval nutritional environment by providing carcasses of different sizes, parents could potentially mitigate against their offspring inheriting a size disadvantage from their inferior developmental environment by rearing disproportionately fewer (and thus correspondingly larger) offspring. However, the reverse was observed: carcass size variation is positively correlated with offspring body size. Intrasexual contest success as adults depended primarily on a body size advantage so this result suggests there ought to be sexual selection for increased body size acting on both sexes. Variation in the post-eclosion nutritional environment had an effect on contest success but this was secondary, being dependent on the relative size of direct competitors. Therefore carcass size predicts body size but the benefits of body size in determining whether an individual becomes a parent or not depends on the social environmental context.

Chapter 4: Male burying beetles extend parental care duration when threats to paternity assurance increase.

In this chapter I wanted to discover if there were quantitative differences in parental care investment depending on variation in the social environment.

In the laboratory the sex-ratio of competitors was manipulated during a reproductive event. I wanted to discover whether males (and/or females) would invest more in parental care when they perceived they had secured a large proportion of genetic parentage of the brood for which they were caring - or whether they were more likely to stay because they risked losing parentage (representation in a brood) if they left early. Males deserted earlier when there was no direct challenge to paternity supporting the hypothesis that males prolonged duration of care not because the brood was likely sired by them (i.e., investing extra care only when offspring are likely to be their own) but in order to increase the probability that offspring will be theirs. Males also stayed for longer when there was competition for their paternity from absent males in the form of stored sperm carried by brood parasitic females. Further support was that caring males increased their mating frequency in response to male competition even though this prolonged the safe interment of the breeding resource. There was no evidence that offspring benefitted from extended male care. The intuitive assumption that males must provide additive benefits of care in a biparental partnership has been challenged in other studies of burying beetles (e.g., Eggert & Sakaluk 1995; Boncoraglio & Kilner 2012) to which is added my finding that male prolonged attendance in a biparental partnership may reduce brood fitness. Females might ultimately prefer to breed without a male in attendance. The benefits conferred by males however may be different in some species where 'takeover' of carcasses already containing larvae (as opposed to usurpation of dominance prior to carcass burial) is more prevalent (*N. orbicollis*: Scott 1990; Trumbo 1991; *N. defodiens*: Trumbo & Eggert 1994; Eggert & Sakaluk 2000). A successful takeover (i.e., involving destruction and replacement of larvae by a conspecific or congeneric burying beetle) was never witnessed during these field studies and wild beetles were

very rarely recorded arriving post carcass-burial (three unsuccessful attempts of which two involved an allospecific beetle that resulted in failure of the brood in approximately 220 witnessed reproductive events in the wild). However, changes in the pre-burial dominance hierarchy is common. From these field studies of *N. vespilloides* the benefit provided by males of protection against takeover seems very unlikely to account for male care in biparental breeding bouts in this species.

In fact, a dominant male is less likely to influence his paternity as a breeding bout progresses (females—at least at an uncontested carcass—may lay the majority of their eggs within 24 hours of arrival at a carcass (Smiseth, Ward & Moore 2006)), but prolonged attendance with frequent mating may still influence a dominant male's future reproductive success, especially if his current reproductive partner finds her next carcass before any other conspecifics. I found that males (and females) in dominant control of a contested carcass are fastidious in eliminating rivals whenever they have the opportunity - males do this by fighting with rival males they encounter near the carcass and by mating repeatedly with females they encounter. Dominant females were almost always rapidly roused to aggression in physical encounters with rival females on the carcass sizes I used. Subdominant (previously defeated) females were always swiftly chased away if ever detected by a dominant female. This contradicts the assumption upon which the design of a recent study was based where two pairs of *N.vespilloides* were introduced to a single mouse carcass and investigators (unable directly to witness agonistic interactions) interpreted results as though all four individuals had bred in a cooperative association (Komdeur *et al.* 2013). In my treatments with 'two pairs' per carcass I did not witness tolerance or cooperative breeding between pairs, rather each individual beetle contested

dominance fiercely by fighting its same-sex rival and subordinate beetles were aggressively denied access to the carcass by the same-sex dominant opponent.

Not only do offspring seem to gain little or no benefit from the male's contribution to biparental care, the result of these social negotiations is that beetles in monogamous pairs (at least on a carcass small enough to be fully utilized by the brood of a single female) desert earlier and leave larger broods.

From the results of this chapter the take home message is that variation in the competitive social environment plays a role, through social interactions, in shaping both patterns of parental investment between sexes and parental performance. Regardless of sex or body size it is beneficial to find an uncontested resource because extra-pair males *and* females both represent a potential threat to the paternity of a resident male.

The importance of body size in direct contests for resources is demonstrated again because in almost all contested treatments, larger beetles defeated smaller same-sex rivals.

Chapter 5: Paternal duties versus mating opportunities: do sex ratio cues affect the duration of male burying beetle parental care?

In this chapter I examined the effect of social environmental variation in a naturalistic setting. I predicted that male potential parents reared with cues providing information about sex ratio might base their decision to care or desert on perceived mating opportunities available elsewhere. In addition his decision might depend on his size (i.e., sexual selection may favour large males due to improved male-male

competitive success and thus access to mates and dominant access to breeding resources).

Sexual selection may operate through female mate choice favouring small males

What I found was that small males attracted proportionately more females than males to carcasses from which they called with pheromones. There is a very strong effect of the first arrival at a carcass on the subsequent arrivals and therefore the constitution of the social environment at a breeding carcass. When a wild female arrives first (to a calling male on a carcass), the likelihood is that a monogamous pair (including the calling male) will result, but if a male arrives first to a calling male, subsequent arrivals and a competitive social environment is more likely. Because smaller males attracted a greater proportion of females it was small males that partnered females in the majority of monogamous pairs. This produced some novel findings. Firstly, sexual selection appears to benefit smaller males via female-choice (or through their greater prowess at attracting females) providing a mechanism by which they avoid competition, but unexpectedly this effect also confers a novel direct benefit for females by reducing the likelihood of female intrasexual competition. Secondly, there is dynamic feedback between the social environment that individuals experience and their own phenotype (mediated by sexual selection). This means that in these beetles the interaction between male-male competition and female mate choice depends on social context which itself is modified by sexual selection. An interesting question raised by these results is if body size is largely determined by carcass size variation (i.e., a non-genetic and/or indirect genetic effect) where does the detectable difference lie between the attributes of males of different size on which females make their choice?

There are still advantages of being large especially in the context of direct contests and, even when contests are infrequent. This advantage for large individuals might fluctuate seasonally during periods of overlapping generations in mid-late summer when frequency and intensity of competition may increase or perhaps in conferring a survival advantage through winter diapause. In the current study 17 out of the 76 calling focal males lost their dominant position on carcasses to wild males, suggesting that there is some scope for investment in traits that increase success in male-male competition.

I also performed an experiment to determine whether there was any pattern of sex or body size differences among wild beetles successfully locating carcasses. Males and females located unoccupied carcasses in similar frequencies but smaller (than average) males find a greater proportion of unoccupied carcasses than do larger males. This experiment also suggested a novel benefit of female mate choice: females faced less intrasexual competition than males because when they arrive in response to a lone male calling they, more often than not, form a monogamous pairing, and in the same way small males benefit by avoiding competition. Perhaps females are also better able to coerce smaller males into ceasing calling (Eggert & Sakaluk 1995). Larger males face more competition than smaller males but they are more likely to succeed against competitors by virtue of being large. An unexplored possible scenario is that on large carcasses beyond the reproductive capacity of a single female, large males might continue calling to attract further beetles. There is evidence in *N. defodiens* that males can benefit from siring a large proportion of the broods of several females (Trumbo & Eggert 1994). One speculative hypothesis for

the maintenance of male uniparental care is that in this specific scenario (i.e., a large male dominates a contested large carcass beyond the maximum egg-laying capacity of any single female) he may be left by the females to care alone for a large brood sired predominantly by him but of mixed maternal origin.

Chapter 6: Individual resource specialisation versus intrasexual competitive success: the effect of body size in a heterogeneous environment.

In burying beetles a carcass simultaneously represents: a) the trophic niche; b) an arena in which sexually selected traits may influence mating success, and c) the vital link between mating success and reproductive success.

Parental performance related to parental body size and carcass size was also examined. The laboratory experiment demonstrated that carcass size determined offspring mean mass independent of parental size (Fig. 6.2b) but that there was an interaction between parental size and carcass size for offspring number successfully reared (Fig. 6.2a). It is clear that differences in the extent and quality of direct provisioning by parents affects offspring survival and rates of growth (Eggert & Müller 1997; Müller, Eggert & Sakaluk 1998; Lock *et al.* 2007; Steiger 2013). However, the results of experiments presented in this thesis (chapters 3 and 6) show that it is not clear at present how important these effects are compared to effects of parental brood tailoring. Laboratory results using artificially controlled brood and carcass sizes can expose variation in parental performance, e.g., Steiger (2013) demonstrated experimentally that offspring size in *N. vespilloides* on carcasses of a standardised size was influenced by a maternal effect (postnatal parental performance) depending on the body size of the caregiver rather than the size of the genetic mother of cross-fostered offspring (i.e., a prenatal maternal effect). However,

in the laboratory experiment described in chapter 6, I found an effect on number, not size, of offspring when initial clutch size was unmanipulated. Furthermore, this effect was robust to carcasses of different size: parents with different body sizes produced offspring that didn't differ in mean size when the carcasses on which their offspring were reared were of similar size. Because the resource is discrete and finite, parents using an inferior resource cannot compensate by increasing provisioning rates on behalf of their developing offspring, as for example occurs in birds (e.g., Tremblay *et al.* 2005; Hinsley *et al.* 2008). However, burying beetles parents adjust brood size to the size of breeding carcass via adjustments to the number of eggs laid (Eggert & Müller 1997) and filial cannibalism of first instar larvae by the male and female parent (Bartlett 1987). This ensures that, although fewer in number, offspring reach a viable adult size and this may help maintain the competitive prowess of brood individuals against those reared on superior resources (Bartlett 1987).

Why don't parents employ effective means to optimise offspring size for competitive ability on carcasses of different sizes? The experiments in this thesis support others that have found *N. vespilloides* does not compensate sufficiently for offspring body size differences caused by carcass size (Bartlett & Ashworth 1988; Eggert & Müller 1997). It has been suggested that parents simply lack accuracy or precision when they tailor broods or that broods outside controlled laboratory conditions will suffer from increased natural attrition that might account for the discrepancy. However, in congeneric burying beetles, *N. orbicollis* (Trumbo & Fernandez 1995) and *N. defodiens* (Trumbo 1990)*,* parents tailor broods sufficiently accurately so that larvae do not differ statistically in mean mass regardless of carcass size differences.

The benefit of being relatively large for burying beetles may depend on population density (Creighton 2005; Steiger *et al.* 2007) and/or competition determined by the ecological community (e.g., other scavengers and congeners). All adults might possess the potential to express the full repertoire of behaviours (i.e., spending time searching for carcasses; calling for females if male; fighting and becoming brood parasites or satellites if displaced from dominant status at a carcass). However, because relative returns from different behaviours are strongly context dependent, interactions between an individual's body size, the phenotypes of other individuals encountered, and unpredictable environmental factors (such as unpredictable carcass availability and size) help maintain wide phenotypic variance.

Non-genetic inheritance?

Chapter 6 presents evidence for individual niche specialisation based on size: large beetle pairs rejected a significant proportion of breeding opportunities on small carcasses. There are some novel implications of this in conjunction with the strong nutritional environmental effect on body size. If beetles tend to select carcasses based on constraints imposed by their own body size, but carcass size (not parental size) mainly determines the mean size of offspring a mechanism for non-genetic stable transgenerational inheritance of a phenotypic trait is generated. This is distinct from indirect-genetic effects that might also play a part in respect of a genetic basis to parental brood tailoring rules. One possibility is that this phenotypic variation generated in part by environmental variation might enable rapid tracking of changeable environments. For example, if the size distribution of available carcasses shifted suddenly (as might happen if an individual dispersed to a new woodland or

via seasonal changes in the availability of carcasses) the size of beetles reared on those carcasses could shift correspondingly in a single generation.

Conclusions:

I have shown that variation in the nutritional environment experienced by larvae of these beetles has fundamental effects on the expression of body size. Body size is the main predictor of success in conflicts for essential breeding resources for males and females but a delay in food availability during the post-eclosion developmental window also affected contest success depending on the size of competitor encountered. Parental performance was preserved, in males and females, over contest success when food was delayed post-eclosion.

A novel insight into a possible motivation for male variation in duration of biparental care is that males remain caring for longer, and mate more frequently when there is competition that threatens their paternity assurance. This threat comes not only from male competitors, but from the stored sperm of absent males who have previously mated with brood parasitic females. This would seem to reinforce the potential advantage held by large individuals.

I have demonstrated potential benefits for small male individuals in their ability to avoid competition through attracting proportionately more females as first arrivals to carcasses that they locate. Small males were not disadvantaged in locating unoccupied carcasses and overall the result of their success in attracting a female before a male led to a higher proportion of monogamous breeding associations involving smaller males. This provides a novel benefit for females thus attracted

because they are more likely enjoy an uncontested carcass on which to reproduce unhindered. In this species there is a dynamic relationship between variation in the social environment and the nutritional environment because carcass size has a direct influence on offspring body size, a trait which influences the likelihood both of meeting competitors and the probability of success when direct contests actually occur.

References:

Badyaev, A. (2008) Maternal effects as generators of evolutionary change. *Annals of the New York Academy of Sciences,* **1133,** 151-161.

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

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

Boncoraglio, G. & Kilner, R.M. (2012) Female Burying Beetles Benefit from Male Desertion: Sexual Conflict and Counter-Adaptation over Parental Investment. *PloS one,* **7,** e31713.

Creighton, J.C. (2005) Population density, body size, and phenotypic plasticity of brood size in a burying beetle. *Behavioral Ecology,* **16,** 1031-1036.

Eggert, A.K. & Müller, J.K. (1997) Biparental care and social evolution in burying beetles: lessons from the larder. *The evolution of social behavior in insects and arachnids* (eds J.C. Choe & B.J. Crespi), pp. 216-236. Princeton University Press, Princeton.

Eggert, A.K. & Sakaluk, S.K. (1995) Female-coerced monogamy in burying beetles. *Behavioral Ecology and Sociobiology,* **37,** 147-153.

Eggert, A.K. & Sakaluk, S.K. (2000) Benefits of communal breeding in burying beetles: a field experiment. *Ecological Entomology,* **25,** 262-266.

Hales, C.N. & Barker, D.J.P. (1992) Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia,* **35,** 595-601.

Hinsley, S., Hill, R., Bellamy, P., Harrison, N., Speakman, J., Wilson, A. & Ferns, P. (2008) Effects of structural and functional habitat gaps on breeding woodland birds: working harder for less. *Landscape Ecology,* **23,** 615-626.

Komdeur, J., Schrama, M.J.J., Meijer, K., Moore, A.J. & Beukeboom, L.W. (2013) Cobreeding in the burying beetle, *Nicrophorus vespilloides*: tolerance rather than cooperation. *Ethology,* **119,** 1138-1148.

Lock, J.E., Smiseth, P.T., Moore, P.J. & Moore, A.J. (2007) Coadaptation of prenatal and postnatal maternal effects. *American Naturalist,* **170,** 709-718.

Müller, J., Eggert, A. & Sakaluk, S. (1998) Carcass maintenance and biparental brood care in burying beetles: are males redundant? *Ecological Entomology,* **23,** 195-200.

Müller, J.K., Eggert, A.K. & Dressel, J. (1990) Intraspecific brood parasitism in the burying beetle, *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Animal Behaviour,* **40,** 491-499.

Saastamoinen, M., Van der Sterren, D., Vastenhout, N., Zwaan, B.J. & Brakefield, P.M. (2010) Predictive adaptive responses: condition-dependent impact of adult nutrition and flight in the tropical butterfly *Bicyclus anynana. American Naturalist,* **176,** 686-698.

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

Smiseth, P., Ward, R. & Moore, A. (2006) Asynchronous hatching in *Nicrophorus vespilloides*, an insect in which parents provide food for their offspring. *Functional Ecology,* **20,** 151-156.

Steiger, S. (2013) Bigger mothers are better mothers: disentangling size-related prenatal and postnatal maternal effects. *Proceedings of the Royal Society B: Biological Sciences,* **280,** 20131225.

Steiger, S., Richter, K., Müller, J.K. & Eggert, A.K. (2007) Maternal nutritional condition and genetic differentiation affect brood size and offspring body size in Nicrophorus. *Zoology,* **110,** 360-368.

Tremblay, I., Thomas, D., Blondel, J., Perret, P. & Lambrechts, M. (2005) The effect of habitat quality on foraging patterns, provisioning rate and nestling growth in Corsican blue tits *Parus caeruleus*. *Ibis,* **147,** 17-24.

Trumbo, S.T. (1990) Reproductive benefits of infanticide in a biparental burying beetle Nicrophorus orbicollis. *Behavioral Ecology and Sociobiology,* **27,** 269-273.

Trumbo, S.T. (1991) Reproductive benefits and the duration of paternal care in a biparental burying beetle, *Necrophorus orbicollis*. *Behaviour,* **117,** 82-105.

Trumbo, S.T. & Eggert, A.K. (1994) Beyond monogamy: territory quality influences sexual advertisement in male burying beetles. *Animal Behaviour,* **48,** 1043-1047.

Trumbo, S.T. & Fernandez, A.G. (1995) Regulation of brood size by male parents and cues employed to assess resource size by burying beetles. *Ethology Ecology & Evolution,* **7,** 313-322.

Victora, C.G., Adair, L., Fall, C., Hallal, P.C., Martorell, R., Richter, L. & Sachdev, H.S. (2008) Maternal and child undernutrition: consequences for adult health and human capital. *The Lancet,* **371,** 340-357.

Appendices:

Appendix 1

General methods:

Outbred laboratory stock was generated from 120 wild beetles collected from Devichoys wood in Cornwall, UK (SW 772 376) during the summer of 2010. Beetles used in our experiments were taken from the F4 generation of this stock. They were maintained in the laboratory at 20°C with an 16:8hr light:dark cycle and housed individually in clear plastic boxes following elosion, until breeding.

Statistics

Parental care was analysed with a generalized linear model, with treatment, sex and carcass mass as fixed effects, using a quasi-binomial error structure. Larval mean mass in the first standardized brood was analysed using a general linear model with treatment (initial delay in feeding), sex (of uniparental carer) and carcass mass as fixed independent variables. Reproductive success was analysed using a generalized linear mixed model with a Poisson error structure. Treatment, sex, breeding bout and carcass weight were fixed terms and beetle i.d. was a random effect in the full model.

Survival was analysed using Cox's proportional hazards with treatment and sex as explanatory variables. In all above analyses a minimal adequate model was determined through stepwise model simplification. Competitive ability was analysed with Fisher's exact tests with a binary win/lose response.

Appendix 1.1

Effect of treatment on beetle mass

Experiment 1

At eclosion, beetles between treatment groups did not differ in size (pronotum width; table 1a) or mass (table 1b) before beginning treatments or in relation to sex. Delayed feeding beetles lost mass during the period of nutritional deprivation, differing significantly from controls after 2 days of starvation (table 1c) and losing 24% of their eclosing mass by the final day of the starvation treatment (day 8) when the mean mass of the delayed feeding group was 64% the mass of controls (table 1d). Three days after the delayed feeding group commenced feeding (i.e., on day 11) there was no longer a significant difference in mass between treatment groups (table 1e). Parity in mass in relation to treatment and sex was maintained until breeding at 21 days post-eclosion (table 1f).

Experiment 2

At eclosion beetles did not differ in size between treatment groups; delayed feeding group mean pronotum width = 4.91 ± 0.05mm, control mean pronotum width = 4.92 ± 0.05mm, (table 2a) or mass, delayed feeding group mean mass = 182.58 ± 5.01mg, control mean mass = 182.08 ± 5.17mg (table 2b). Experimental treatment groups differed significantly in mass after 8 days; delayed feeding group mean mass = 131.03 ± 3.48mg, control mean mass = 211.73 ± 5.33mg (table 2c), but consistent with the first experiment, final (breeding) mass was not statistically significantly different between treatments; delayed feeding group mean mass = 209.88 ± 4.72mg, control mean mass = 207.8 ± 4.98mg (table 2d).

Appendix 1.1. cont:

Table 1: Two-way ANOVA comparisons of adult beetle size and mass in relation to treatment and sex. a) pronotum width; b) parent beetle mass at eclosion; c) mass at 2 days post-eclosion; d) mass at 8 days post-eclosion; e) mass at 11 days post-eclosion; f) adult beetle breeding mass (21 days post-eclosion).

	d.f.	MS	*F*	*P*
a) Beetle size				
Treatment	1	0.004	0.047	0.829
Sex	1	0.058	0.690	0.409
Treatment:Sex	1	0.202	2.416	0.124
Error	75	0.084		
b) Mass at eclosion				
Treatment	1	157.2	0.168	0.683
Sex	1	9.3	0.010	0.921
Treatment:Sex	1	1951.2	2.101	0.151
Error	75	928.8		
c) Mass at 2 days post-eclosion				
Treatment	1	5554	5.052	0.028*
Sex	1	1	0.001	0.976
Treatment:Sex	1	1257	1.144	0.288
Error	75	1099		
d) Mass at 8 days post-eclosion				
Treatment	1	141762	158.978	<0.0001***
Sex	1	3	0.003	0.955
Treatment:Sex	1	928	1.040	0.311
Error	75	892		
e) Mass at 11 days post-eclosion (3 days after feeding the delayed feeding group)				
Treatment	1	392.6	0.284	0.596
Sex	1	189.6	0.137	0.712
Treatment:Sex	1	883.0	0.638	0.427
Error	75	1383		
f) Breeding mass (21 days post-eclosion)				
Treatment	1	7.1	0.006	0.938
Sex	1	829.3	0.714	0.401
Treatment:Sex	1	252.7	0.217	0.642
Error	75	1161.9		

Appendix 1.1. cont:

Table 2**:** Two-way ANOVA comparisons of adult beetle size and mass in relation to treatment and sex. a) beetle size at eclosion (pronotum width); b) beetle mass at eclosion; c) mass at 8 days post-eclosion; d) mass at time of experiment (21 days post-eclosion).

	d.f.	MS	*F*	*P*
a) Beetle size at eclosion				
Treatment	1	0.000	0.001	0.971
Sex	1	0.054	0.576	0.450
Treatment:Sex	1	0.000	0.003	0.954
Error	76	0.094		
b) Mass at eclosion				
Treatment	1	5.0	0.005	0.946
Sex	1	9.8	0.009	0.924
Treatment:Sex	1	2.4	0.002	0.962
Error	76	1063.8		
c) Mass 8 days				
Treatment	1	130250	157.187	<0.0001***
Sex	1	2	0.002	0.963
Treatment:Sex	1	211	0.255	0.615
Error	76	829		
d) Mass 21 days				
Treatment	1	86.1	0.091	0.763
Sex	1	1776.6	1.884	0.174
Treatment:Sex	1	25.3	0.027	0.870
Error	76	943		

Appendix 2

'Nicrocosm' contest arenas:

Nicrocosms were constructed from 400mm lengths of black plastic tubing ø110mm with three ø40mm × 50mm ports at compost-level designed to allow beetle entry and egress. For use in the laboratory each nicrocosm was enclosed in a 30L plasterer's bucket within which was 2cm of moist compost (Fig. A.1). A mouse carcass was placed on the compost in each nicrocosm. All above ground activity on and around the carcass, was filmed and recorded with closed-circuit, infra-red surveillance cameras, equipped with infra-red light-emitting diodes (N08CX night vision CCTV camera) mounted in each nicrocosm. Commercially available DVR software and video surveillance software permitted 24 hour recording (AVerMedia NV6240 Express, DVR version 7.7.0.0007; www.avermedia-dvrs.com).
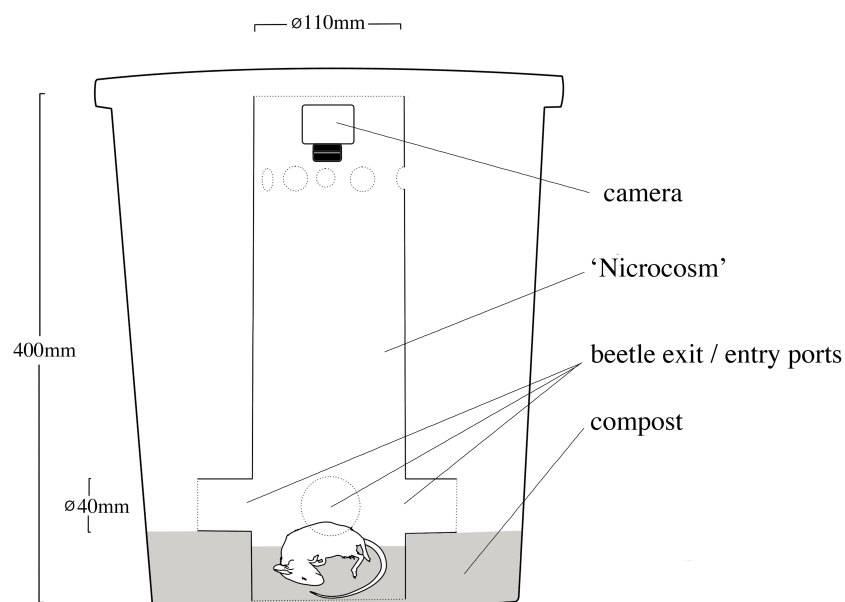

Figure A.1: Cut-away diagram of an arena designed to enable burying beetle contests to be observed and recorded (in the central, cylindrical 'Nicrocosm') in the wild. Subordinate (defeated) beetles are able to leave the immediate vicinity of the carcass. The laboratory set-up is depicted here with the Nicrocosm cylinder enclosed in a 30L plasterer's bucket to retain any deserters.