Phenotypic Plasticity in Female Burying Beetle's: Individual Responses in a Multidimensional Environment

Submitted by Patrick Alan Kitchener to the University of Exeter as thesis for the
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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.

Summary

Phenotypic plasticity is defined as the environmentally sensitive expression of alternative phenotypes produced within a single genotype. The majority of research on plasticity has been demonstrating its evolutionary implications, specifically highlighting how plasticity can drive, direct and constrain genetic evolution and how plasticity will evolve. However, our current understanding of plasticity and evolution is limited by a lack of empirical studies investigating individuals and how multiple environmental variables interact influencing the direction or the degree of the plastic response. Yet, this is important to consider in future research as population level responses are determined by how individuals respond, furthermore by studying individuals rather than populations we can quantify the extent of variation amongst individuals in which selection acts upon. Studying the response on a multivariate environment also has significant benefits if we want to improve our knowledge of phenotypic plasticity. This is because generally studies focus on manipulating only one environmental variable, yet interactions between environments alter the benefits and costs of plasticity/the optimal response to environmental change, thus potentially changing the direction or the magnitude of selection acting on plasticity.

In this thesis, I address this gap by investigating how individuals in a multivariate environment respond to environmental change using burying beetles (*Nicrophorus vespilloides*) as our model system. Burying beetles are considered highly plastic, largely because they reproduce exclusively on carrion, a resource that is unpredictable in space and time, meaning the environment is never consistent between breeding attempts. The aim of this thesis was therefore to explore further and in more detail, how burying beetles respond to environmental change and to investigate the importance and potential benefits of plasticity in burying beetles. Additionally, this thesis also aimed to answer two overlooked questions in research. Firstly, looking at how females respond to changes in the competitive environment and variation in resource quality, predicting that plasticity in maternal behaviour would aim to maximise offspring traits (i.e. offspring size) in a competitive environment. And secondly, assessing how individual age affects the plastic response to reproductive resource quality, in which I expected our data to follow theoretical predictions that it would be adaptive for old individuals to be unresponsive.

The results in chapter 2 revealed that parental behaviour, whether it being direct care (feeding offspring) or indirect care (carcass maintenance), significantly declined in a competitive environment, following more closely with a costly competitive hypothesis. This suggests that competitive stress limited the level of energetic resources available for maternal investment, and that evolution between the traits is potentially negatively correlated. However, contrary to the above result, the chapter also found that female burying beetles adjusted brood size adaptively, by reducing offspring number and increasing larval mass in a competitive environment, thus matching offspring phenotypes with the environment, a form of adaptive transgenerational plasticity.

In chapter 3 I demonstrate that to a certain extent plasticity is age-dependant. It was found that, on average, old individuals do not adjust their reproductive traits in relation to the size of the breeding resource (carrion), while young individuals do. However, this pattern in response is only illustrated within certain environmental conditions, as the chapter found that the results above are dependent on a population's current and previous breeding environment. The chapter also reveals that there is substantial variation in the plastic response amongst individuals. Specifically illustrating that old individuals are responsive, demonstrating that studying at a population level doesn't provide sufficient information to fully understand how individuals are responding to the environment. This result would therefore indicate that while age may have some effect on influencing the plastic response to environmental change, shown at a population level, it is only likely to play a minor role influencing plasticity, as individuals of the same age substantially differed in their plastic response.

In this thesis, I conclude by discussing how the results have improved our understanding of plasticity in female burying beetles and how selection may be expected to act on plasticity in such a species given our results. I also talk about how the theses has improved our broader understanding of phenotypic plasticity and how it may develop research for the future.

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Chapter 1: General Introduction

1.1 Introduction to Phenotypic Plasticity

Individuals can adapt to environmental change by altering the phenotypic expression of traits, providing a fitness advantage in the new environmental conditions, often referred to as phenotypic plasticity (Pigliucci, 2001). Phenotypic plasticity is typically defined as the environmentally sensitive expression of alternative phenotypes produced within a single genotype (Pigliucci, 2001). Whether plasticity is adaptive or non-adaptive depends upon whether the response increases or decreases the individual's fitness. Although at first, any form of environmentally-induced phenotypic variation (adaptive or non-adaptive) was regarded as non-heritable and considered only evolutionary noise (Simpson, 1953; Williams 1966; West-Eberhard, 2003) which slowed the rate of adaptive evolution (Levin, 1988). It is now widely considered that phenotypic plasticity plays an integral role in evolutionary processes (Baldwin, 1902; Ghalambor, McKay, Carroll, & Reznick, 2007; Ghalambor *et al*., 2015; Pfennig *et al*., 2010; Pigliucci, 2001; Pigliucci, 2007; Price, Qvarnstrom, & Irwin, 2003; West-Eberhard, 2003; Wund, 2012).

Phenotypic plasticity has been demonstrated to be ubiquitous across morphological, physiological, life-history and behavioural traits and expansive across the animal kingdom (Pigliucci, 2005; West-Eberhard, 2003). The pattern of response is determined by both an individual's genetic architecture but also their current environment and/or their previous experience (Schlichting & Pigliucci, 1998; Piersma & Drent, 2003; West-Eberhard, 2003). Predominately, any plastic response has been categorised as either developmental (non-reversible) or activational (reversible) (Pigliucci, 2001; West-Eberhard, 2003). The latter, activational plasticity is an innate response triggered by an environmental stimulus and reversible when the stimulus dissipates (Piersma & Drent, 2003)(e.g., Creighton, Smith, Komendat, & Belk, 2015). Whilst developmental plasticity is described as when the environment influences the developmental trajectory of a phenotype (West-Eberhard 2003)(e.g., Losos *et al*., 2000). The major difference between the two is the trade-off between phenotypic integration and response time, thus both are favoured under different environmental contexts (review, Snell-Rood, 2013).

At first phenotypic plasticity was generally studied as a response to abiotic environments, for example, how different populations of *Arabidopsis thaliana* dealt with wind, demonstrating that wind significantly effects the degree of branching within the plant (Pigliucci, 2002). However, studies on plasticity have now been extended to include changes in internal state, ecology and the social environment. This is because state variables such as age and nutritional quality will favour plasticity, as an individual's optimal trait trajectory is state-dependant (Houston & McNamara, 1992). For example, the quality of nutritional environment as larvae affects the developmental trajectory of adult horn morphology, an illustration of developmental plasticity based on state variables (Moczek, 1998). Ecological changes have also now been considered as a plastic response, for example in tiger snakes (*Notechis scutatus*) they adaptively adjust growth in jaw morphology in relation to prey size density (Aubret *et al.,* 2004; Aubret & Shine, 2009). Changes in the social environment however have been considered less often but are increasingly being studied in relation to plasticity. For example, studies have manipulated the parental environment during reproduction, to analyse male and female plastic responses in parental care and investment, which has led to key hypothesis on social evolution to be tested, such as how biparental care can be an evolutionary stable strategy (Creighton *et al*., 2015).

Plasticity has therefore become a central topic in multiple fields of research (e.g., Hendry, 2016; Miner, Sultan, Morgan, Padilla, & Relyea, 2005; Pfennig *et al*., 2010). This importantly includes conservation research, in order to analyse how populations will deal with the increasing threat of global climate change (Chevin *et al.,* 2010; Vedder *et al.,* 2013). However, it is the role plasticity plays in trait evolution that has attracted extensive theoretical and empirical research. With the central focus on evaluating how adaptive plasticity may evolve, as well as studying the ecological and evolutionary implications plasticity may have (see section 1.2).

1.2 Importance of Understanding Plasticity in Evolution

Such is the importance of plasticity in evolutionary processes, researchers have called for an extension of the evolutionary synthesis (Pigliucci, 2007). This is because plasticity can not only evolve itself, influencing the level of phenotypic variation in a population, but it can also influence genetic evolution (see section 1.2.1). Baldwin (1902) first noted the implications plasticity may have on evolution and while it was largely overlooked at that time, research on plasticity and evolution has grown dramatically over the recent past, such that, plasticity is now thought to play a major role in species diversification and speciation

(Pfennig *et al.,* 2010). Below I discuss the main hypotheses around the two major roles plasticity is regarded to play in evolution and why it is growing in importance within literature.

1.2.1 Phenotypic Plasticity and the Implications for Genetic Evolution

The most referred to example of how plasticity effects genetic evolution is termed the Baldwin effect, which suggests that plasticity will drive adaptive evolution in a novel environment. Baldwin (1902) proposed that when populations experience novel environmental change, plasticity can permit populations to persist until natural selection can then act on the variation. Thus, an individual's plastic response would drive the evolution of a phenotype in the new environment (Ghalambor *et al*., 2007; Pigliucci, Murren, & Schlichting, 2006; Price *et al*., 2003; West-Eberhard, 2003). This process is also thought to influence the direction of evolution. This is because plasticity will determine the phenotypic expression within the novel environment which potentially reflects previous cryptic genetic variation, altering the heritable phenotypic variance and shifting the phenotypic means in a population (Foster, 2013; Ghalambor *et al*., 2007; Lande, 2009; Price *et al*., 2003; West-Eberhard, 2003). In a similar manner plasticity is thought to effect genetic evolution by genetic assimilation. This process differs from the Baldwin effect as it is expected that the phenotype expressed in the new environment will become a canalised phenotype (no plasticity) (e.g., Aubret, 2015; Aubret & Shine, 2009).

Currently, it is unknown how common either phenomenon is within nature (Crispo, 2007). Furthermore, it remains debated as to whether plasticity truly plays a role in evolution and whether it facilitates or constrains evolution (De Jong, 2005; Ghalambor *et al*., 2007). However, theory has suggested that the effect of plasticity on genetic evolution is dependent on how the phenotype in the new environment relates to the optimal trait (Ghalambor *et al*., 2007; Price *et al*., 2003). For example, if the plastic response to the new environment is the optimal trait, then plasticity would shield genetic variation from selection and consequently constrain genetic evolution of the phenotype.

Additionally, there are common assumptions made for both processes/hypothesis, however past research has shown that even if assumptions aren't met, plasticity is still known to impact genetic evolution. For example, it is often assumed that the plastic trait is heritable.

Yet, even if the trait is not heritable, plasticity is still proposed to have implications for associated traits, by altering the environment of other phenotypes (West-Eberhard, 2003; Pfennig *et al.,* 2010). Evidence for this has been reported in *Anolis sagrei* lizards, where they demonstrated that a behavioural plastic response to move to an arboreal habitat in the presence of a predator consequently altered the selective pressures acting on leg length (Losos *et al.,* 2004). A second assumption is that the phenotypic response to environmental change is adaptive and therefore provide an advantage in the new environment and selected upon, ultimately leading to altering the direction and speed of evolution (West-Eberhard, 2003; Pfennig *et al.,* 2010). However, maladaptive plasticity is still regarded as having implications on evolution assuming the trait is heritable and that there is sufficient genetic variation, as it will favour individuals with little or no plasticity and therefore drive directional selection, meaning populations in different environments may converge to the same phenotypes (Ghalambor *et al*., 2007; Grether, 2005). This has been referred to as genetic compensation and has been empirically supported in Trinidadian guppies (*Poecilia reticulate*) (Ghalambor *et al.,* 2015).

1.2.2 Evolution of Phenotypic Plasticity

If plasticity is adaptive, an individual's response would maintain a higher fitness across the environmental gradient and therefore would be under natural selection and evolve according to theory (Baldwin, 1902; Houston & McNamara, 1992; Pigliucci, 2001, 2005; Schlichting & Pigliucci, 1998; West-Eberhard, 2003). Whether selection acts on plasticity or is indirectly selected for, by selection favouring extreme phenotypes of the response remains debated (Scheiner & Lyman, 1989; Schlichting & Pigliucci, 1998; Via, Gomulkiewicz, & Jong, 1995). Either way it can potentially result in plasticity being under selection and evolve.

Numerous models have consequently analysed the conditions that favour phenotypic plasticity. One of these is the degree of environmental variation that occurs over space and time (Murren *et al.,* 2015). Selection is expected to favour plasticity in heterogeneous environments, where different phenotypes will prosper in different environments and where the environment varies either within or across generations (Bradshaw, 1965; Via & Lande, 1985; Berrigan & Scheiner, 2004; Murren *et al.,* 2015). Furthermore, it is predicted that the rate of evolution in plasticity will then depend on the frequency of environmental change and the diversity of environments experienced (e.g., Scheiner, 1993; Sultan & Spencer, 2002). For example, if a population experiences only a rare novel environment we are instead likely to see a plastic response driving genetic evolution as the Baldwin effect suggests, rather than directional selection acting on plasticity. The evolution of plasticity is also dependant on the costs and benefits of the plastic response (Moran, 1992; DeWitt & Scheiner, 2004; Auld *et al.,* 2010; Murren *et al.*, 2015). A cost of plasticity refers to the decline in fitness due to maintaining a highly plastic genotype compared to a less plastic genotype (Dewitt *et al*., 1998). Thus, whether plasticity is evolutionary adaptive relies on the costs incurred by maintaining and/or producing the response. Furthermore, it is argued that the evolution of plasticity is also dependant on the reliability of environmental cues (Scheiner & Holt, 2012) and is constrained by variation in selection pressures (Snell-Rood *et al.,* 2010). Lastly, models assume that populations are genetically variable, as a lack of genetic variation will impose constraints on evolution (Schlichting & Pigliucci, 1998; Murren *et al.,* 2015).

While the focus has been understanding and modelling how plasticity evolves, less is known about the broader implications adaptive plasticity may have. Yet, if plasticity is selected for, it could potentially influence the level of control a genotype has over phenotypic expression as the phenotype is becoming more environmentally dependant. Selection on plasticity also potentially means that sub-optimal phenotypes evolve. For example, a non-plastic individual may have a higher fitness in environment A but because the individual doesn't respond adaptively to environment B, the plastic individual has a greater fitness when measured across both environments, thus selection would favour plastic individuals despite plastic individuals having a sub-optimal phenotype in environment A. Furthermore, it is argued that selection on plasticity will have an effect on both direct and indirect ecological interactions (Miner *et al.,* 2005). For example, plants adjust the growth of roots in relation to the concentration of nutrients in the soil to maximise nutrient foraging, however this response directly affects the level of competitive interactions amongst plants (Hodge, 2004). Lastly, its suggested that selection on plasticity can lead to ecological and evolutionary traps, as the environmental cue stimulating the response overtime may become unreliable (Miner *et al.,* 2005).

1.3 - Current Gaps in our Understanding

Despite the substantial growth in research around the subject of plasticity and strong current level of knowledge of how plasticity evolves there are certain aspects of research still overlooked and unclear.

Currently, we have a strong understanding of how populations respond to environmental change, however we know less about how individuals differ in their plastic response (IxE) (Nussey, Wilson, & Brommer, 2007). This is despite population level responses being determined by how individuals respond. Furthermore, to fully understand how natural selection acts on plasticity, it requires analysing IxE as selection acts on individual variation, which is the result of both genetic and non-genetic differences that are present amongst individuals (Nussey *et al*., 2007). Consequently, analysing IxE allows us to assess natural selection on plasticity, as the relationship between lifetime fitness and the plastic response to environmental change within an individual can be directly analysed (Nussey *et al*., 2007). This has been done in a population of red deer (*Cervus elaphus*). The study illustrates that individuals significantly differ in their plastic response and that variation in early life conditions partly describes this variation. Additionally, they show selection is acting on this variation with plasticity being favoured. However, they also found that individuals did not follow population level responses and that selection on plasticity is dependent on early life conditions/individual state differences, highlighting the importance of looking at individual responses (Nussey, Clutton-Brock, Elston, Albon, & Kruuk, 2005). This follows theoretical predictions in which it is suggested the optimal level of plasticity is state dependant (Houston & McNamara, 1992). Thus, understanding why individuals differ is important to establish the direction of selection and why the evolution of plasticity may differ between populations. This remains a growing area of research but will only improve our understanding of plasticity.

Another important element which has only recently progressed is to consider how multiple environments interact (Westneat, Stewart, & Hatch, 2009). Most empirical work has only analysed the effect of an environment independently and linearly. For example, house sparrows (*Passer domesticus*) are known to adjust provisioning behaviour to nestling age, brood size and partner effort. However, these factors also interact, with the interaction between nestling age and brood size raising questions as to what this means in terms of the evolution of parental behaviour, and what parents are actually responding to (Westneat, Hatch, Wetzel, & Ensminger, 2011). Similar patterns have been demonstrated in multiple species and phenotypes (e.g., Relyea, 2004; Stillwell, Wallin, Hitchcock, & Fox, 2007; Westneat *et al*., 2009). Considering multidimensional/variate environments is therefore of importance as it potentially means that the evolution of plasticity to a certain environment may differ between populations, due to another environment interacting with the response (e.g., Nussey *et al*., 2005). In this thesis when using the words multidimensional and multivariate environments it is therefore referring to analysing traits across multiple environments (i.e. social environment and size of breeding resource), the variation within these environments (i.e. the degree of competition) and how the environmental variables interact (i.e. social environment x size of breeding resource).

Analysing the consequences of phenotypic plasticity has also attracted comparatively little research. Yet according to theory we expect plasticity to have major ecological and evolutionary implications (Baldwin, 1902; Miner *et al*., 2005; Pigliucci, 2001; West-Eberhard, 2003). Currently, research has only broadly highlighted its potential implications, however, it is now argued to gain a comprehensive view of the consequences of plasticity, there needs to be a holistic approach in future research, studying at a species, population and individual level. Furthermore, there is a need to establish whether you’re interested in studying the effects of variation in plasticity, or the effects due to a greater level of inter-individual phenotypic variation due to selection acting on plasticity. Methodology to test such approaches however have yet developed (Forsman, 2015).

1.4 - Measuring Plasticity: The Reaction Norm Framework

The way a phenotype is expressed across an environmental gradient is described as a reaction norm (Pigliucci, 2001). Typically, they are visualised by plotting the environmental gradient (either continuous or discrete) on the x axis against the phenotypic value, where the line would then describe the norm of reaction. Assuming the relationship between the environment and phenotype is linear, reaction norms can then be statistically analysed by the coefficients of a linear regression. Consequently, they are characterised by two parameters, elevation and slope and can be viewed as quantitative traits (Schlichting &

Pigliucci, 1998; Pigliucci, 2001). The elevation demonstrates the mean-level of phenotype expressed within that environment, whilst the slope illustrates the phenotypic response to environmental change, in other words the level of phenotypic plasticity. Classically, this approach was first applied at a genetic level, to assess variation among genotypes in how they respond to environmental change, commonly referred to as genotype-by environment interaction (GxE) (Via & Lande, 1985; De Jong, 2005). This was broadly to investigate the level of genetic variation in plasticity and consequently its evolutionary potential (Scheiner & Lyman, 1989; Via & Lande, 1985). The technique also provided a way of understanding how the parameters co-vary, for instance whether genotypes displaying a higher phenotype are more plastic. This same structure is now applied at a species, population and individual level (Dingemanse & Wolf, 2013; Nussey *et al.,* 2007).

Reaction norms have also provided a way to estimate directional selection on plasticity. If reaction norm parameters and a suitable measure of fitness (e.g. lifetime reproductive success) have been measured independently, the direction and intensity of selection can then be statistically assessed by analysing the association between an individual's (or a genotype's) elevation, slope and their fitness. This provides us with a direct measure of selection on both parameters and their correlation (Lande & Arnold, 1983; Weis & Gorman, 1990). In a similar manner, if a measure of fitness is available, it is suggested that reaction norms can also be used to visualise the fitness costs associated with a plastic response and can be quantified with the same statistical methods (Auld *et al.,* 2010; Murren *et al.,* 2015). Furthermore, we can use reaction norms to study the evolution of plasticity and how plasticity may impact genetic evolution, through assessing whether there is a change in slope and elevation between past and present populations (Nylin & Gotthard, 1998; Schlichting & Pigliucci, 1998; West-Eberhard, 2003).

1.5 – Plasticity in Burying beetles (*Nicrophorus vespilloides*)

Burying beetles provide an ideal model system to look at phenotypic plasticity in further detail. This is because the ecological resource needed for reproduction varies both spatially, temporally and the quality of the resource is unpredictable. Consequently, these beetles experience variation in one or multiple environmental conditions during reproduction and because of this are regarded as highly plastic. Furthermore, the complexity of their behaviour amongst invertebrates which has attracted extensive research means they are an excellent model to investigate key evolutionary and behavioural questions on plasticity in parental care and life-history traits.

Commonly referred to as sexton beetles, burying beetles belong to the family Silphidae. They are characterised by their unique life-history where they reproduce exclusively on decaying animal matter. Once carrion is discovered, using chemosensory (Conley 1982), beetles first remove fur or feathers and apply anal secretions to delay decomposition before burying it underground (Scott, 1998). Eggs are then laid around nearby soil and hatch into altricial larvae which move into the carcass crypt (a brood chamber on the carcass) (Scott, 1998). Larvae will then feed of the carcass for approximately a period of 3-4 days before dispersing (Scott, 1998). During this time one or both parents will provide parental care improving offspring growth and survival (Eggert, Reinking, & Müller, 1998; Scott, 1998). Currently, through manipulating carcass quality, research has illustrated the importance of plasticity in multiple traits and the implications it has on reproductive success to adapt to resource quality (table 1). For example, burying beetles respond to the quality of the resource by investing further into current reproduction, producing more offspring on larger carcasses (Creighton, 2005; Creighton, Heflin, & Belk, 2009; Smith, Creighton, & Belk, 2015; Trumbo & Fernandez, 1995). As a result, larger carcasses are associated with a larger brood size and consequently a greater reproductive success, and is therefore regarded as an adaptive response (Creighton, 2005; Trumbo & Fernandez, 1995). However, because papers have not studied individual variation we currently cannot determine whether individuals are maximising their lifetime reproductive success.

Burying beetles have not only been shown to respond to carcass size, evidence shows that both males and females respond to changes in the social environment (table 1). The majority of social interactions in both males and females in burying beetles occur during two main phases, competition and parental care. In both scenarios, plasticity again is seen as evolutionary adaptive in certain contexts. For example, parents will adjust parental effort in relation to their partner, by compensating for any decline in the level of care provided (Creighton, Smith, Komendat, & Belk, 2015; Rauter & Moore, 2004; Smiseth, Dawson, Varley, & Moore, 2005), as predicted if biparental care is to evolutionary stable (Houston & McNamara, 1999). Thus, plasticity in parental investment is argued to have a role in the evolution of biparental care, assuming the fitness benefits of responding outweigh the costs associated with parental care and the potential loss in future reproductive opportunities. Adapting to individual state factors is also a major part in burying beetle's lifestyle due to their unique life-history. Substantial evidence has therefore been collected evaluating adjustments in phenotypic expression in relation to individual state (table 1), something theoretically expected if individuals are following their optimal trait trajectory (McNamara & Houston, 1996). For instance, as individual's age it is generally predicted that they should increase their investment into current reproduction (Williams, 1966; Clutton-Brock, 1984), a theory supported in literature on burying beetles (Creighton *et al*., 2009). Nutritional quality and prior experience have shown to have similar effects on trait expression (table 1).

Despite our strong current understanding of the importance of plasticity in burying beetles, with multiple studies highlighting so, our knowledge of plasticity in burying beetles still remains incomplete. Firstly, this is because recently there has been development in theory and suggested changes in methodology. It is now argued that to study plasticity we need to look at how individuals and/or genotypes respond rather than only at population level (Nussey *et al.*, 2007). Whilst this has been done in some cases (e.g., Carter *et al*., 2015) it hasn't in others. Secondly, although many environments have been tested, the addition of analysing plasticity on a multidimensional level has meant there still remains much to be explored about plasticity. Especially because current evidence has found that multiple environments are known to independently influence the same trait. Lastly, because of the stochasticity of the reproductive resource there are many independent environments that are yet to be tested. For example, few studies in burying beetles, as well as the broad literature have looked at female-female competition and how individuals respond to this social environmental change. Yet, it seems highly important if we want to further establish how sexual or social traits are selected upon and evolve in females as it has done in research studying sexual traits in males.

Table 1, Summary table of the published studies illustrating the current evidence of plasticity in burying beetles.

Response variable	**Empirical Evidence**
Responding to Carcass Size/ Resource Quality Change	
Parental Behaviour	(Smiseth & Moore, 2002)
Life-History Strategy	(Billman, Creighton, & Belk, 2014; Creighton, Heflin, & Belk, 2009)
Aggressive Behaviour	(Eggert & Müller, 1992)
Reproductive Investment	(Creighton, 2005; Creighton, Heflin, & Belk, 2009; Smith, Creighton, Belk, *et al.*, 2015; Trumbo & Fernandez, 1995)
Responding to Social Environment Change	
Male Mating Rate	(Male density - Hopwood, Moore, Tregenza, & Royle, 2015)
Reproductive Tactics	(Male density - Carter, Head, Moore, & Royle, 2015)
Parental Behaviour	(Male density - Hopwood *et al.*, 2015), (Partner effort - Smiseth, Dawson, Varley, & Moore, 2005; Smith *et al.*, 2015), (Brood size - Rauter & Moore, 2004), (Offspring begging - Smiseth & Moore, 2002)
Responding to Individual State Factors	
Parental Behaviour	(Male age - Benowitz, Head, Williams, Moore, & Royle, 2013)(Female age - Lock, Smiseth, Moore, & Moore, 2007)(Previous experience of contest - Pilakouta, Halford, Rácz, & Smiseth, 2016)
Life-History Strategy	(Female age - Cotter, Ward, & Kilner, 2011; Creighton *et al.*, 2009)(Previous experience of carcass size - Billman *et al.*, 2014)
Mating Rate	(Male age - Benowitz et al., 2013)
Competitive Behaviour	(Female age - Trumbo, 2009; Trumbo, 2012)(Nutritional state - Hopwood, Moore, & Royle, 2013)(Male Social experience - Lee, Head, Carter, & Royle, 2014)
Reproductive Investment	(Female Nutrtional state - Steiger, Richter, Müller, & Eggert, 2007)
Reproductive tactics	(Male Nutritional state - Hopwood *et al.*, 2013)
Adult Body Size	(Nutritional state - Hopwood, Moore, & Royle, 2014; Trumbo & Xhihani, 2015)

1.6 – Thesis Aims and Objectives

This thesis aims to combine the recently established concepts of analysing individual level plasticity within a multivariate environment, to investigate further how burying beetles respond to environmental change and what the implications are on their reproductive success. It was also to broadly interpret whether and how selection may be acting on plasticity within certain contexts and further our knowledge of what plasticity is, using burying beetles as our model system. As discussed, this is fundamentally important as detailed research on both plasticity and burying beetles is limited, despite being a major part of their life history and plasticity being expansive across the animal kingdom. Additionally, other than these broader aims, in this thesis I wanted to test/answer some key independent questions that are currently overlooked within the subject.

In Chapter 2 of my thesis I tested how females plastically responded to competition. Intraspecific interactions in burying beetles are often intense and common between females as the reproductive resource is unpredictable (Scott, 1998; Wilson & Fudge, 1984). However, we currently know little about the effects competition has on reproductive performance in females, and whether this reproductive cost could be defined as an adaptive plastic response, through intergenerational plasticity. Yet, if we are to understand how plasticity evolves to social environmental change and the influence it may have on social evolution we need to understand how individuals are responding to social environmental change and if this response differs between environments, for instance the reproductive resource size.

In Chapter 3 of my thesis I investigated whether plasticity was age-dependant. It is theoretically expected that the optimal level of plasticity is state-dependant but few have empirically explored such cases (Houston & McNamara, 1992; Fischer *et al.,* 2014). Yet it could potentially alter evolution, as the costs and benefits of plasticity alter with a change in state. In this study, I consequently analysed if a burying beetle's response to carcass size was affected by its age. Furthermore, by manipulating age and carcass size, which have both previously been shown to impact life-history and reproductive investment strategies, it allowed us to investigate how reproductive investment decisions in a burying beetle are made, and whether plasticity is truly beneficial throughout an individual's lifetime.

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Chapter 2 – How do Females Respond to Competition?

2.1 Abstract

The expression of social traits is shaped partly by the social interaction that occurs between and amongst other individuals. However, because individuals differ in trait expression, the social interaction that occurs amongst individuals varies, thus the optimal response to the social environment changes and consequently plasticity is thought to be beneficial. Studying social plasticity to understand the evolution of reproductive and competitive traits has consequently become important, but research has mostly concentrated on males. The importance of plasticity in female social interactions is therefore largely unknown, however it is fundamental if we want to establish why sexual/social traits are selected upon (or not). To address this gap, we examined how females respond to competition. Specifically, this chapter investigated at how female-female competition affects reproductive investment, and how this response varies with resource quality and amongst individuals using burying beetles as our model system. This chapter found that unlike other species, burying beetles did not adaptively adjust parental behaviour and instead investing in competition had costs on female parental investment. Consequently, this result would suggest that evolution between these traits is likely to be negatively correlated. However, this chapter did demonstrate that female beetles adaptively manipulated offspring brood number so that offspring were larger in a competitive environment. This could be a form of adaptive transgenerational plasticity that will potentially evolve in order to match offspring phenotypes with the current environmental conditions.

2.2 Introduction

Investigating social interactions, such as competition, using new research methods are fundamental if we are to develop the social and sexual selection theories (Darwin, 1871; West-Eberhard, 1983). The strength and direction of selection on social traits is partly shaped by how an individual’s phenotype is influenced by the phenotype of another or multiple individual’s (indirect genetic effect) (Moore, Brodie III, & Wolf, 1997; Wolf, Brodie III, Cheverud, Moore, & Wade, 1998). However, social environmental pressures on phenotypic expression are highly variable, the fitness of a phenotype fluctuates with the social environment, consequently favouring plasticity (Schlichting & Pigliucci, 1998; Pigliucci, 2001). Plasticity is the ability of a single genotype to express multiple phenotypes in relation to environmental change (Pigliucci, 2001) . It allows individuals to adjust phenotypic expression in relation to the optimal response determined by a social interaction, thus maintaining fitness across the environmental gradient (Baldwin, 1896; Houston & McNamara, 1992; Pigliucci, 2001). For example, the ability to compensate for partner desertion during parental care means reproductive performance is maintained when a partner deserts offspring (McNamara *et al.,* 1999). Even though social plasticity is potentially adaptive it has only recently attracted interest (Dingemanse, Kazem, Réale, & Wright, 2010; Westneat, Hatch, Wetzel, & Ensminger, 2011). Yet, it can have implications for evolutionary processes: either by (1) driving, directing and constraining genetic evolution (Price *et al.,* 2003; Foster, 2013), or (2) Plasticity itself can be under selection, consequently influencing how a genotype is translated into a phenotype (DeWitt *et al.,* 1998; Pigliucci, 2005).

Individuals compete over access to essential resources needed for survival and reproduction (e.g. mating opportunities, food and territory). The fitness of an individual is therefore partly dependent on the competitive phenotypes expressed by conspecifics (IGE) (Andersson, 1994; Moore *et al.,* 1997). Generally, increasing the expression of competitive traits (e.g. ornaments, aggression and weaponry) increases access to the limited resources and improves individual reproductive success (Darwin, 1871; Andersson, 1994). However, increasing investment into competitive traits results in both a lower future reproductive potential and/or a decline in current reproductive effort (van Noordwijk & de Jong, 1986; Roff, 1992; Stearns, 1992). Despite this growing literature in competition and analysing the consequences of their response, few have looked at how these responses to changes in the competitive environment relate to studies on phenotypic plasticity. Yet the intensity of competition varies between individuals and across environments, meaning plasticity is potentially adaptive (McNamara *et al.,* 1999; Pigliucci, 2001; Patricelli *et al.,* 2002). This is because plasticity would mediate the costs and benefits of competitive traits in relation to the environmental context, maximising reproductive success (van Noordwijk & de Jong, 1986; Houston & McNamara, 1992; Sinervo & Svensson, 1998; Patricelli *et al.,* 2002). Currently the majority of research on this topic has focused on males (Patricelli *et al.,* 2002;

Carter *et al.,* 2015; Herczeg *et al.,* 2015; Kuczynski *et al.,* 2016; Nandy *et al.,* 2016). For example, male *D. melanogaster* plastically adjust copulation duration and aggressiveness to social environmental change (number of rivals) to minimise the fitness consequences incurred, leading to the potential for selection to act on plasticity (Nandy *et al.,* 2016).

Research on how females respond to competition however has received less attention until recently. The majority of work has been highlighting that an increased investment into competition would reduce a females investment into current and/or future reproduction (Trivers, 1972; West-Eberhard, 1983; Fitzpatrick *et al.,* 1995; Clutton-Brock, 2009; Cain & Ketterson, 2013; Sartori *et al.,* 2015). Yet alternatively, females may actually be adaptively reducing offspring number as to maximise offspring size in a harsh environment (increased competition) (Fox & Mousseau, 1998; Mousseau & Fox, 1998). This response is defined as an adaptive maternal effect which ensures offspring phenotype matches environmental conditions (Fox & Mousseau, 1998; Mousseau & Fox, 1998; Allen *et al.,* 2008; Bentz *et al.,* 2013; Inzani *et al.,* 2016). However, there still remains much to be understood about the plastic response to competition in females. Few papers have looked at post-natal investment plasticity as an adaptive maternal effect to competition. Even though offspring that receive increased parental care often have increased growth, leading to more exaggerated expression of sexually selected traits, improving competitive ability (Wolf, Brodie, & Moore, 1999). Secondly, little is known as to how multiple environments influence responses. For example, studies have demonstrated how resource quality or food abundance influences the intensity of competition (Grant, 1993; Dubois *et al.,* 2003) but we don’t know what the wider implications are for parental care and reproductive investment

This chapter attempts to address these gaps, by testing how females plastically adjust post-natal parental care and reproductive investment to the level of competition experienced and whether this plasticity depends on the value of the breeding resource. It also aimed to look at how competition effects brood mass and mean larval mass within a brood as to answer whether the response is a cost due to competitive stress or instead an adaptive maternal effect. Furthermore, we aim to analyse the variation in response amongst individuals.

In this study I used *Nicrophorus vespilloides*, a species of burying beetle which reproduce exclusively on the carcasses of small vertebrates, an unpredictable but highly valued resource (Scott, 1998). As a result, intraspecific and interspecific competition is common in both males and females (Scott, 1998; Wilson & Fudge, 1984). Typically, *N.vespilloides* breed on carcasses of small vertebrates <30g and competition can be intense, especially on relatively large carcasses (15g+) (Pukowski, 1933; Bartlett & Ashworth, 1988; Eggert & Müller, 1992). Reproductive success on contested carcasses is highly skewed in favour of the dominant individual (Eggert, Otte, & Müller, 2008; Müller, Eggert, & Dressel, 1990). Attaining dominance mainly involves aggressive fighting, described as beetles using their mandibles to bite their opponent’s leg in an antiparallel position until an individual begins retreating (Pukowski, 1933), this can be severe with partial or total loss of limb (Müller et al., 1990; Safryn & Scott, 2000). Generally a larger body size reflects dominance (Otronen, 1988; Safryn & Scott, 2000). Previous research has demonstrated that a burying beetles body size is partly determined by the level of post-hatching maternal care and the relationship between the size of the carcass and brood size (Bartlett & Ashworth, 1988; Creighton, 2005; Steiger, 2013; Trumbo, 1992). This is because *N. vespilloides* provide elaborate parental care, including direct regurgitation of food to offspring, improving offspring growth and survival (Eggert, Reinking, & Müller, 1998; Scott, 1998). Additionally, parents also adjust brood size by filial cannibalism and altering the number of offspring laid and consequently, parents can influence the relationship between brood size and carcass size (Bartlett, 1987; Müller & Eggert, 1990). How a female burying beetle responds to an environmental change can consequently have major implications on an offspring’s phenotype and both its reproductive success and that of the offspring.

It was predicted that if females reduce their levels of maternal care in a competitive environment then this would indicate effects of competitive stress, while if they upregulated their parental care and offspring larval mass increased the results would fit the adaptive plastic response hypothesis. Similarly, it was predicted that if individuals maintained or there was a decline in brood size and mean larval mass remained constant or declined, it would demonstrate that competition is costly on reproduction. Alternatively, if a decline in brood size was found along with an increase in mean larval mass then it was predicted that females are maximising offspring phenotype through adaptive transgenerational plasticity. Furthermore, it was expected that individuals would substantially vary in their response irrelevant of whether the data supports either hypotheses. However, more specifically it was predicted that if data followed a costly competitive hypothesis then individuals experiencing increased level of aggression would incur a greater cost on reproductive traits. Lastly, we predicted that carcass size would influence the response and it would differ depending on how they respond to competition. If it is the prior hypothesis that competition is costly, it is expected that the effects would be exaggerated because competition will increase on larger carcasses, thus having a greater negative affect on reproductive traits. If instead the data follows the adaptive plastic response hypothesis then it was predicted that carcass size would not influence the response, but instead would only influence the level of variability found amongst individuals, in which on larger carcasses more variation will be found as differences in individual quality are exaggerated.

2.3 Methodology

General methodology and maintenance

Experimental stock population of *N. vespilloides* were attained from a population of 50 males and 50 females, caught from Devichoys Wood, Cornwall, UK (N50°11’47”E5°7’23”) during August 2015. Outbred populations were maintained by randomly breeding virgin individuals in individual breeding boxes (17X12X6cm), filled with 2cm of damp soil and a mouse carcass (15-25g; Livefoods Direct, Sheffield). Larvae were subsequently placed into individual rearing containers after dispersal (7X7X4cm) and kept in incubators 21°C (±2°C) with a 16L:8D hour cycle. After eclosion individuals were fed twice weekly on two mealworms (*Tenebrio*) for a period of 20 days before experimental trials. All experimental beetles remained virgin individuals with no social experience prior to the experiment. During sexual maturation beetles were weighed and the pronotum width was measured three times and averaged using digital calipers (to 0.1mm). Each female beetle in the experimental stock were then marked to allow instantaneous identification during behavioural observations. Marking was completed by lightly scratching the dorsal surface in one of four locations on their distinctive orange patches (front left, front right, back left and back right), and then applying black nail varnish. Marking has been shown to have no detrimental effect on behaviour (Hopwood *et al.,* 2013).

Before being randomly placed into treatments, experimental beetles were pre-mated by randomly pairing males and females, which were left for 24h to ensure sufficient fertilisation. Pre-mating was completed to remove male effects and biologically relevant as almost all (93%) of females caught in the wild were found to have been mated (Müller and Eggert, 1989). Consequently, they have the opportunity to breed on their own or just compete with other females. Once mated, males were kept aside and not involved any further in the experiment until they were re-mated before the second breeding attempt.

Experimental design

We used a repeated measures design allowing us to look at individual responses, where focal female beetles were measured across two breeding attempts, either a) by themselves or, b) after a competitive environment with another pre-mated female. Breeding attempts were sequential with 3 days between the end of the first and start of the second breeding attempt. At random, half the experimental population began with the competitive environment followed then by the non-competitive environment, while the opposite occurred for the other half to control for order effects. This also allowed us to analyse how individuals adjust their current reproductive investment vs future reproductive investment when experiencing a different environment in their first breeding attempt as well as controlling for order effects. We also manipulated carcass size (mean±SD), a resource necessary for reproduction, with half being given a large carcass (22.64±0.403) and half a small carcass (11.05±0.39) throughout the entire experiment. Carcass size did not differ between breeding attempts (Paired t-test, t=1.77, df=69, P=0.081), minimising any behavioural effect due to the available resource (Jenkins *et al.,* 2000). In total, it meant we had 4 experimental treatments (C-competitive environment, NC- Non-competitive environment); a) C-NC, large carcass, b) NC-C, large carcass, c) C-NC, small carcass and d) NC-C, small carcass.

Competitive assay

After pre-mating, focal females were randomly paired with same age non-focal females and the pronotum size difference was recorded because of the relationship between size and dominance (Safryn & Scott, 2000). This method is preferred over size matching as it replicates conditions they are likely to experience in the wild and ensures variation in fighting ability (Hsu *et al.,* 2006). The appropriate size carcass was then placed in a breeding box (17X12X6cm), filled with 2cm of damp soil. At the onset of the scotophase (the start of the dark phase in a light and dark cycle) both females were then simultaneously placed into the box, reducing the effects of carcass ownership on contest behaviour (Otronen, 1988). Competitive bouts were then filmed for a period of 20h, by placing a nicrocosm (400mm length of black PVC-U Ø 110mm) within the breeding box, centralising the mouse. Exit holes to the exterior then allowed individuals to leave this contest area freely throughout the time frame. Within the nicrocosm an infrared surveillance camera (N08CX night vision CCTV camera) was placed, which used motion detection software (AverMedia NV6240 Express, dvr version 7.7.0.0007; www.avermedia-dvrs.com) to record competitive behaviour (Further details, see Hopwood *et al.*, 2013). The number and duration of aggressive interactions between the females was recorded to measure the intensity of competition for the reproductive resource. Dominance was then assigned to the individual who began chasing the other of the carcass and initiated in prenatal carcass preparation (Hopwood *et al.*, 2015). After the 20 hours, the non-focal female was removed, whether it was a dominant or sub-ordinate individual. The nicrocosm was removed and the breeding boxes were relocated back in to the incubators at 21^{o}C. We removed the non-focal female as we wanted to assess the female's response to competition without the effect of parasitism on a focal females reproductive success (Eggert & Müller, 1992). Oviposition typically occurs 24-48 hours after coming into contact with a carcass (Müller & Eggert, 1990). By removing the non-focal female within this time meant we could be sure that all offspring belonged to the focal individual and why a period of 20h was chosen for competitive observations. Because we could not assign dominance before experimental trials, it meant our focal females varied between dominant and sub-ordinate.

Parental care assay

We measured parental behaviour to analyse the effects of a competitive environment on maternal investment. Before recording parental behaviour individuals were first acclimatised in red light conditions with lids removed, for a period of 30 minutes as previously suggested (Smiseth, Dawson, Varley, & Moore, 2005). After acclimation, instantaneous scan sampling was used to observe behaviour (Martin & Bateson, 1993), recording the behaviour every 1 minute for 30 minutes (Smiseth & Moore, 2004). Observations of parental care were measured in four periods, approximately 24, 30, 40 and 48 hours after larval hatching, where parental care is at its peak of intensity (Smiseth, Darwell, & Moore, 2003), an essential period for improved larvae success and size *(Eggert et al*., 1998). Parental behaviour was recorded as direct care, the regurgitation of carrion to offspring (Smiseth, 2004), or, indirect care, described as adding secretions to the carcass (Carcass maintenance/ processing) and displaying on top of the carcass (Guarding) (Scott, 1998; Smiseth, 2004). Observations were recorded using infrared surveillance cameras (N08CX night vision CCTV camera) to minimise disturbance, similar to methodology above for competitive assay. We placed the camera into a nicrocosm (400mm length of black PVC-U Ø 110mm) within the breeding box. We then used motion detection software (AverMedia NV6240 Express, dvr version 7.7.0.0007; www.avermedia-dvrs.com) to record parental behaviour (For further details, see Hopwood *et al.,* 2013).

Once larvae had dispersed, offspring number and brood mass were then measured as a proxy for fitness and reproductive success (Rauter & Moore, 2004a). Lastly, female body mass was measured before competition, after competition and after larval dispersal during each breeding attempt.

Statistical analysis

Population responses- In order to analyse the effects of social environment on female parental care and life-history traits, I performed linear mixed models in R version 3.0.2 (R Core Team 2015) using package lme4 (Bates *et al.,* 2015). In the models, it included carcass size, order, beetle size and the interaction between carcass size and social environment as fixed effects. The interaction allowed me to assess if resource quality influenced decisions on competitive interactions. Female ID was also included as a random effect. To achieve the minimum adequate model, I used a likelihood ratio test to compare models, starting with interaction terms, until only significant terms remained ($P<0.05$). The package lmertest (Kuznetsova *et al.,* 2015) was then used to obtain model summary information. This method was used for the following response variables; number of offspring, mean larval mass and brood mass. To assess female parental care, we used a general linear model with same fixed effect structure but the model had a binomial error structure. The model had a random effect structure with female ID (1|ID) and an observation level effect (1|observation), which is a method used to account for overdispersion (Harrison, 2014). Significant fixed effect terms were achieved using the likelihood ratio test starting with interaction terms.

Individual responses- To assess whether females on average varied significantly in their trait expression I tested the random effect, female ID. The significance of female ID was assessed using likelihood ratio test once non-significant fixed effects were removed from the model and were done for all measured traits. Using package ASReml (Gilmour *et al.,* 2009) I investigated how phenotypic variance (conditional on the fixed effects) is distributed across our two traits in separate environments in both resource quality environments. This was achieved by a bivariate analysis of a mixed model where each row in our data structure represented an individual, and contains the observation/dependant variable in both a competitive and non-competitive environment. To determine significant terms I then compared models with different (co)variation structures using the likelihood ratio test (Visscher, 2006). The first model had a single variance term, where there was no covariance and the variance is constrained to be the same across the social environments. We then compared this to a model allowing different variance estimates for a competitive and non-competitive environment. A significant result would indicate individual plasticity assuming measurement error to be equal across environments. This is because this result would indicate unequal variances across environments meaning individuals must vary in how they respond to environmental change. Finally, the second model was compared against an unstructured covariance model, meaning traits could co-vary as well as allowing separate variance terms for the different social environments. This result indicated whether there was significant individual covariance across environments: the correlation across environments can then be calculated using $COV(_{E1,E1})/\surd(V_{E1}*V_{E2})$. The correlation term indicates the strength and sign of the covariance – for example, a strong positive term indicates that it is generally parallel across environments (such that individuals that perform well in one environment also perform well in the other). Bivariate models were run separately for each carcass size treatment by sub-setting the data to again analyse differences in individuals due to resource quality.

2.4 Results

The effects of competition on female post-natal investment

The interaction between carcass size and social environment was non-significant for all three parental traits (table 1). Carcass size had no effect on the level of parental care provided (table 1). The order in which the social environment was experienced did not influence the level of care given (table 1). Larger beetles provided the same level of parental care (table 1). However, the social environment in all three traits was significant (table 1). Specifically, our result suggested more time was spent providing parental care, as well as direct and indirect, when there was no female competition for the reproductive resource (figure 1).

Table 1, Mixed model analyses testing plasticity in parental care to social environment change. Significant terms of the fixed effects were obtained using likelihood ratio test following stepwise removal of terms.

Total time spent providing parental care (%)		
Fixed effects	**$\chi2_1$**	**P value**
Social environment*Carcass size	2.812	0.093
Social environment	15.973	**<0.001**
Carcass size	0.12	0.728
Order	0.067	0.795
Beetle size	0.328	0.566
Total time spent providing direct care (%)		
Social environment*Carcass size	4.283	0.112
Social environment	9.228	**0.002**
Carcass size	0.051	0.821
Order	<0.001	0.986
Beetle size	0.199	0.655
Total time spent providing indirect care (%)		
Social environment*Carcass size	1.465	0.226
Social environment	25.851	**<0.001**
Carcass size	0.335	0.551
Order	0.009	0.921
Beetle size	0.244	0.621

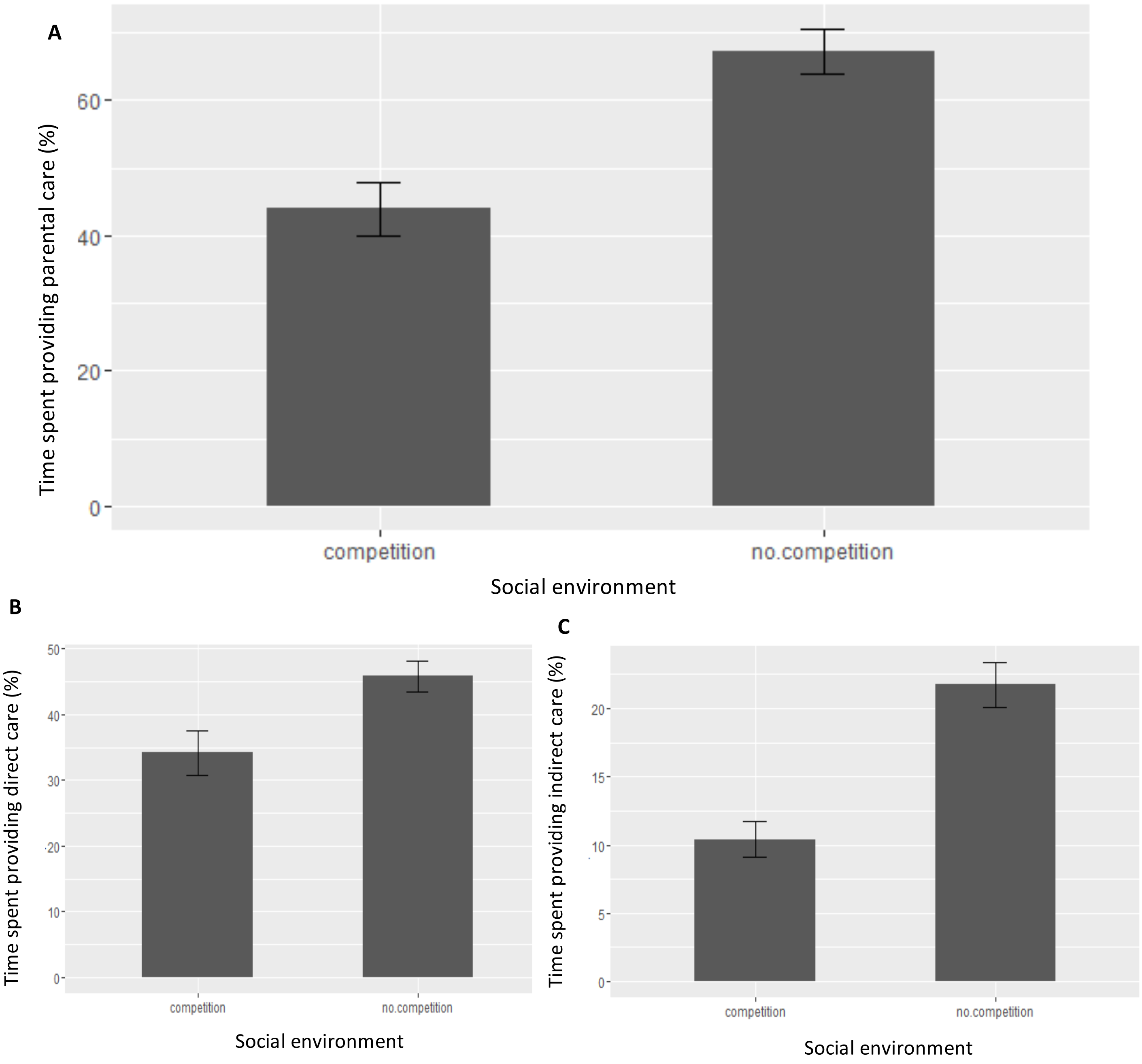

Figure 1, illustrates comparisons (mean±1SE) of the time spent on three parental care traits measured across the two different social environments (competition/no competition). (A) total parental care, (B) direct care (or provisioning offspring) and (C) indirect care (or carcass maintenance). It indicates that all traits measured show a similar pattern, the average level of parental care in a population is reduced in a competitive environment. However, it also demonstrates that the extent of the decline in care is greater in the level of indirect care provided compared to the level of direct care.

The effects of competition on female reproductive/ life-history traits

The interaction between carcass size and social environment was non-significant in all three traits (table 2). However, I found a significant main effect of social environment on all traits (table 2). Females produced significantly more larvae in a non-competitive environment (figure 2a, 7.6±0.84 more) and had a greater brood mass at dispersal (figure 4a, 0.47±0.13g greater). However, in a competitive environment larvae were significantly heavier (figure 3a, by 0.043±0.005g). There was also a significant main effect of carcass size in the number of offspring produced and the brood mass (table 2). On larger carcasses more offspring were produced (figure 2b, by 4.1±0.98) and there was a greater brood mass (figure 4b, 0.85±0.19g). This effect of carcass size was consistent across the social environment, meaning the population level response to social environmental is identical across the two carcass size environments, illustrated in figure 2c and 4c. Mean larval mass was not influenced by carcass size (table 2, figure 3b) but was influenced by beetle size, with larger beetles producing heavier offspring (table 2). Order had no effect in any of the traits (table 2).

Table 2, Mixed model analyses testing the effect of social environment change on reproductive and life-history traits. Significant terms of the fixed effects were obtained using likelihood ratio test following stepwise removal of terms.

Number of offspring		
Fixed effects	**$\chi2_1$**	**P value**
Social environment*carcass size	0.933	0.334
Social environment	57.461	**<0.001**
Carcass size	15.528	**<0.001**
Order	0.214	0.643
Beetle size	0.003	0.955
Mean Larval mass (g)		
Social environment*carcass size	3.207	0.073
Social environment	57.918	**<0.001**
Carcass size	1.209	0.271
Order	3.123	0.077
Beetle size	13.327	**<0.001**
Brood mass (g)		
Social environment*carcass size	0.193	0.659
Social environment	11.653	**<0.001**
Carcass size	17.726	**<0.001**
Order	0.925	0.336
Beetle size	2.815	0.093

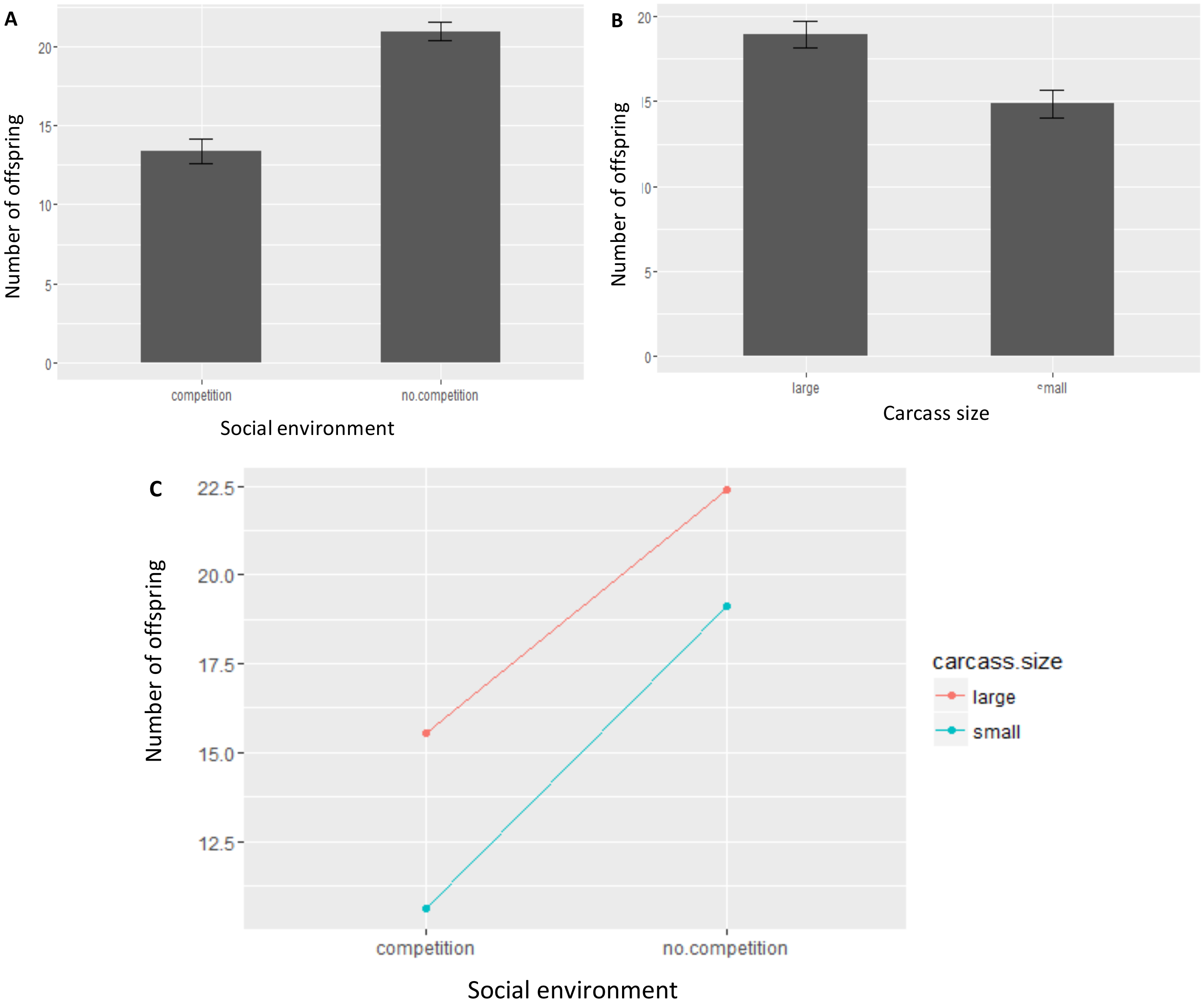

Figure 2, focuses on how changes in the environment alter the average number of offspring produced within a brood within a population. **2a** showing the effect of the social environment on the number of offspring produced (mean±1SE), **2b** showing the effect of carcass size on the number of offspring produced (mean±1SE) and **2c** demonstrating how the interaction between the above mentioned environmental variables influence the number of offspring (mean). It highlights that the social environment and carcass size have independent effects on offspring number (2a and 2b) but there is no interaction, as shown by the almost parallel gradients between the lines (2c).

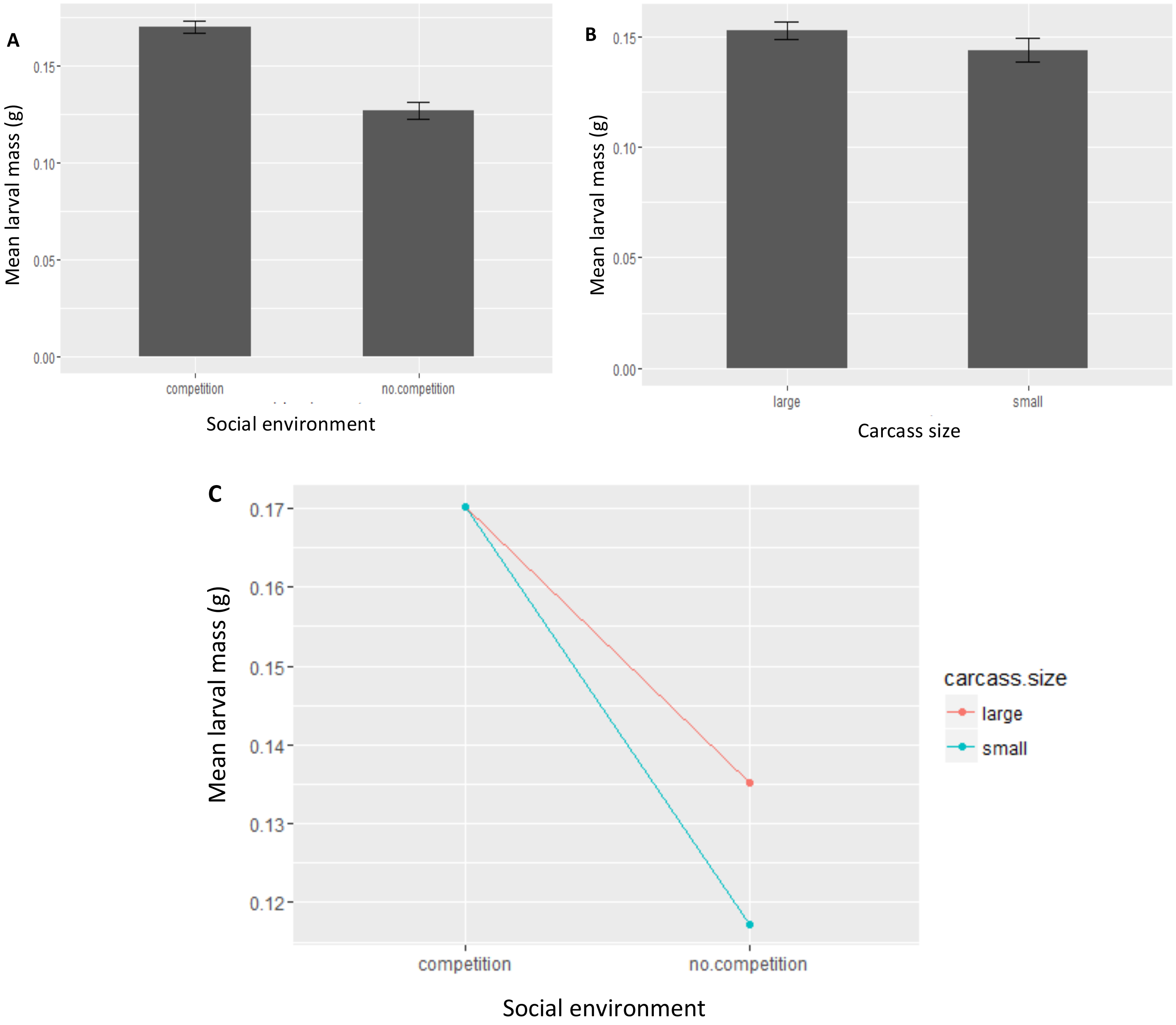

Figure 3, focuses on how changes in the environment alter the mean larval mass produced within a brood. **3a** demonstrating the effect of the social environment on the mean larval mass (g) (mean±1SE), **3b** showing the effect of carcass size on the mean larval mass (g)(mean±1SE) and **3c** showing how the interaction between the above mentioned environmental variables influence mean larval mass (g). The graphs highlight a potential interaction between the environmental variables, as shown by the difference in gradient between the lines (2c), however after statistical analysis it was found to be non-significant. However, figure 2a does show that changes in the social environment did have an impact on the reproductive trait, mean larval mass was higher in a competitive environment.

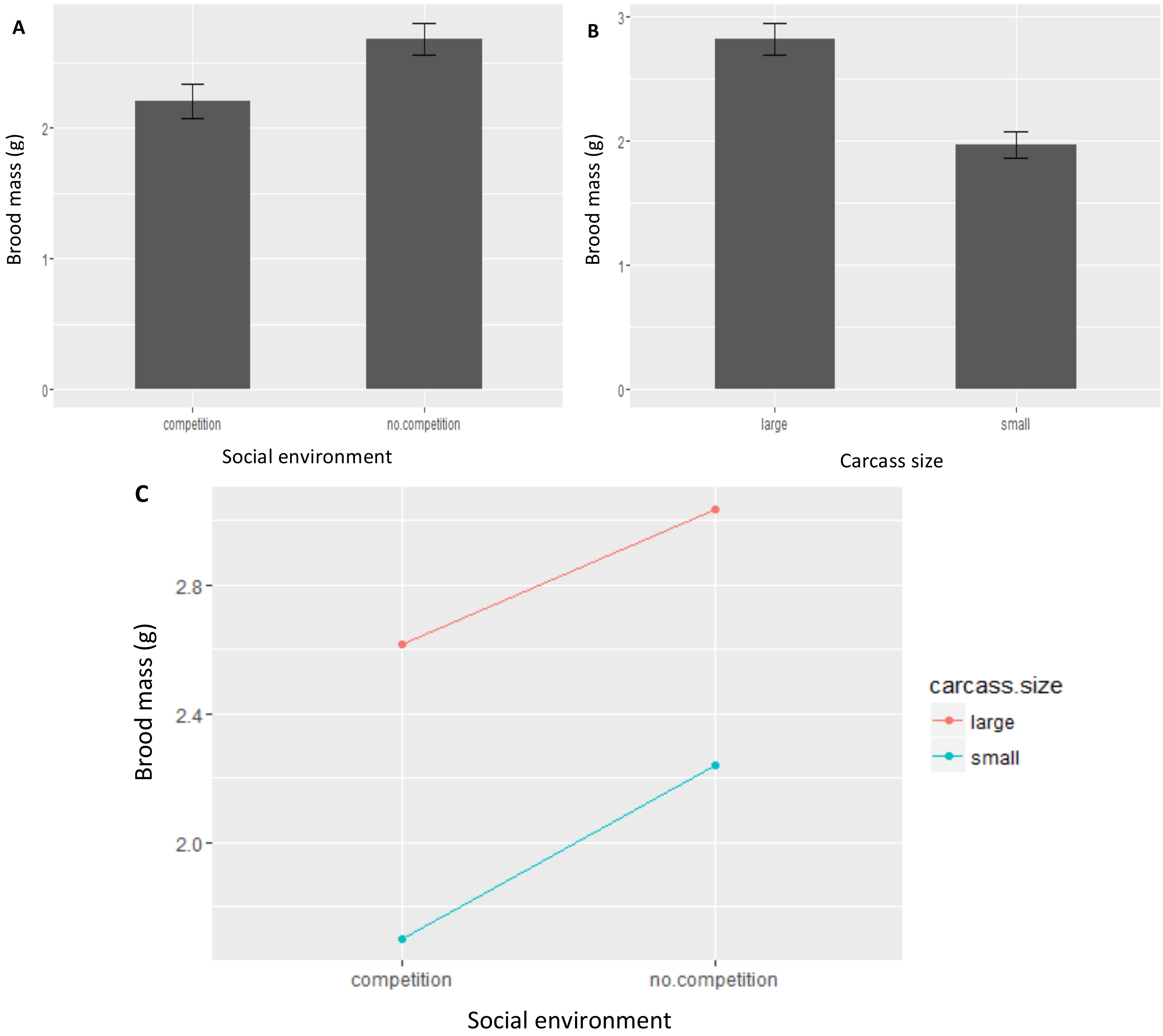

Figure 4, focuses on how changes in the environment alter the populations average brood mass (g). **4a** showing the effect of the social environment on the brood mass (g) (mean±1SE), **4b** showing the effect of carcass size on brood mass (g) (mean±1SE) and **4c** demonstrating how the interaction between the above mentioned environmental variables influence brood mass (g) (mean). It highlights that the social environment and carcass size have independent effects on offspring number (4a and 4b) but there is no interaction, as shown by the almost parallel gradients between the lines (4c).

Is there variation amongst individuals in their response?

Female ID significantly improved the fit of the model for the response variable brood mass (table 5). The average brood mass produced therefore significantly differed between individuals. However, all parental care measures (total, $\chi2_1$=2.076, P=0.149/ direct, $\chi2_1$=0.048, P=0.826/ indirect, $\chi2_1$=0.716, P=0.397) and life-history traits (table 3 and 4) were otherwise non-significant.

The units of measurement used to quantify parental effort and the limitations imposed by the model restricted the ability to measure individual plasticity (IxE) in parental behaviour, but individual responses are illustrated in figure 5a, indicating IxE. All reproductive traits however fit model assumptions for analysing IxE and were run in a bivariate model and shown graphically in figure 5. The results demonstrate that the number of offspring individuals produce is more variable in a competitive environment and on a large carcass (table 3). However, the variance in the number of offspring does not differ between social environments on either size of carcass in addition to there being no significant covariance. This suggests that generally individuals responding to the social environmental change, show a similar pattern in response with other individuals and there is little variation in plasticity (IxE). This is true on both sized carcasses. Individual responses for mean larval mass are shown in figure 5c. The results indicate that only on a large carcass there is variation in how individuals plastically respond to the social environmental change (figure 5c, table 4 - Vc=Vnc), but there is no pattern in the response, as those that produced larger offspring in a non-competitive environment did not necessary produce heavier offspring in the competitive environment (figure 5c, table 4 – Covariance). Lastly, the results demonstrated that the change in overall brood mass to social environmental change differed between individuals (IxE) on both sized carcasses, indicated by a significant covariance but a weak correlation value (table 5). The result is graphically illustrated in figure 5d.

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Table 3, demonstrates the significance of the random effect Female ID in the linear mixed model using likelihood ratio test. The table also shows the bivariate analysis of a mixed model to interpret whether plasticity varies between individuals, in which we used the likelihood ratio test to test if models significantly differed.

Model		**Individual plasticity**				
Response- Number of offspring	**Female ID**	**Variance (competition) Vc**	**Variance (no competition) Vnc**	**Vc=Vnc**	**Co - variance**	**R±SE**
Full population	$\chi2_1$=1.52, P=0.2	-	-	-	-	-
Large carcass	-	45.92±10.76	28.74±6.75	$\chi2_1$=1.99, P=0.078	$\chi2_1$=2.01, P=0.077	0.231 ±0.156
Small Carcass	-	26.28±6.95	14.38±3.82	$\chi2_1$=2.59, P=0.053	$\chi2_1$=0.12, P=0.36	0.065 ±0.186

Table 4, demonstrates the significance of the random effect Female ID in the linear mixed model using likelihood ratio test. The table also shows the bivariate analysis of a mixed model to interpret whether plasticity varies between individuals, in which we used the likelihood ratio test to test if models significantly differed.

Model		**Individual plasticity**				
Response- Mean larval mass (g)	**Female ID**	**Variance (competition) Vc**	**Variance (no competition) Vnc**	**Vc=Vnc**	**Co - variance**	**R±SE**
Full population	$\chi2_1$=0, P=1	-	-	-	-	-
Large carcass	-	0.0004± 9.12e-05	0.0012± 2.76e-05	$\chi2_1$=10.86, P<0.001	$\chi2_1$=0.024, P=0.43	0.026 ±0.165
Small Carcass	-	0.0011± 2.91e-04	0.0008± 2.19e-04	$\chi2_1$=0.58, P=0.223	$\chi2_1$=1.5, P=0.11	0.226 ±0.177

Table 5, demonstrates the significance of the random effect Female ID in the linear mixed model using likelihood ratio test. The table also shows the bivariate analysis of a mixed model to interpret whether plasticity varies between individuals, in which we used the likelihood ratio test to test if models significantly differed.

Model		**Individual plasticity**				
Response- Brood mass (g)	**Female ID**	**Variance (competition) Vc**	**Variance (no competition) Vnc**	**Vc=Vnc**	**Co - variance**	**R±SE**
Full population	$\chi2_1=8.54$, P=0.003	-	-	-	-	-
Large carcass	-	1.43±0.34	1.11±0.26	$\chi2_1=0.59$, P=0.221	$\chi2_1=3.48$, P=0.03	0.3±0.15
Small Carcass	-	0.48±0.13	0.52±0.14	$\chi2_1=0.03$ P=0.43	$\chi2_1=5.42$, P=0.01	0.42±0.155

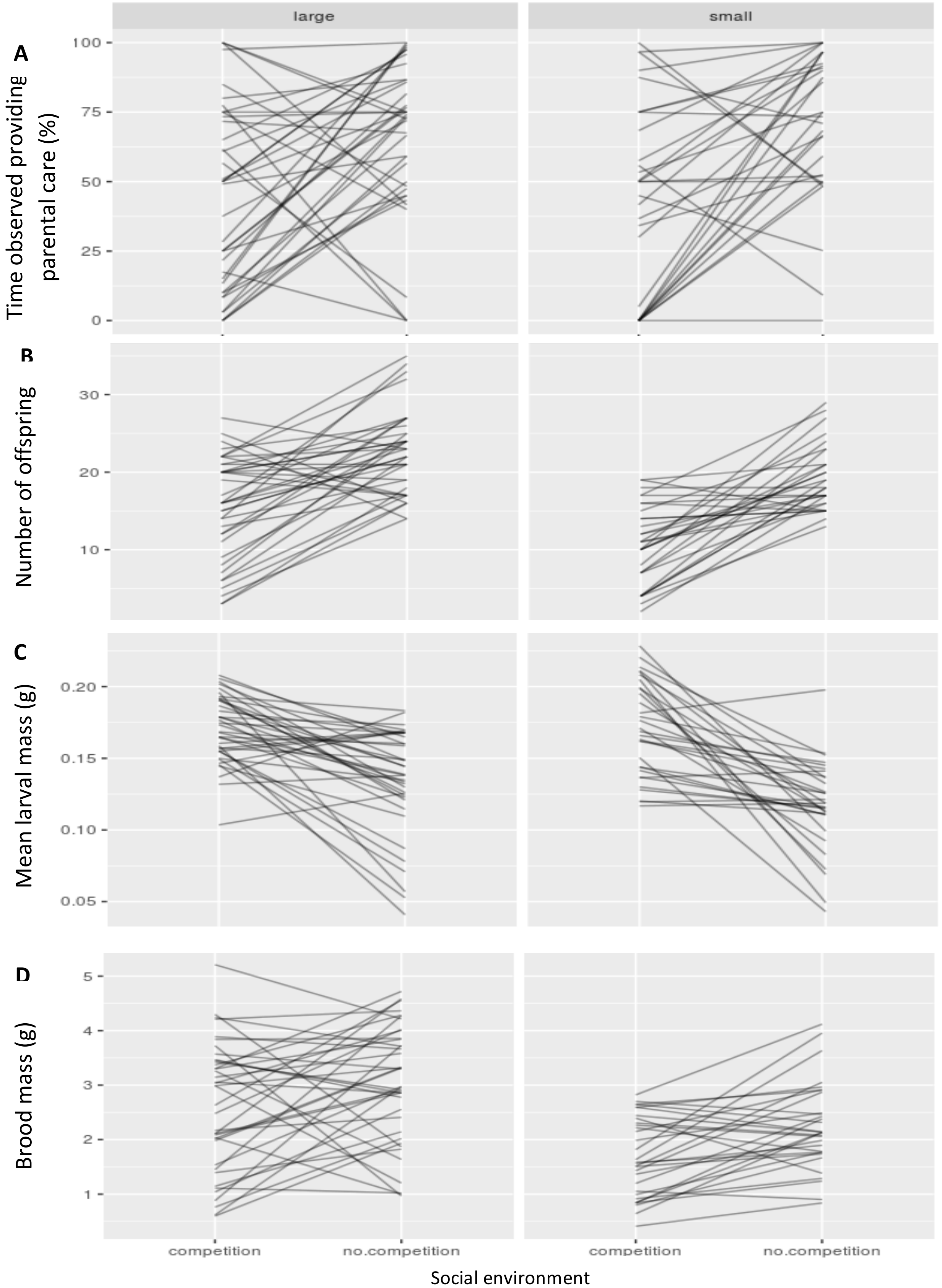

Figure 5, demonstrates the individual responses and phenotypic expression in relation to the social environment. Each line therefore represents an individual's plastic response for: **A)** Total level of parental care (%), **B)** Number of offspring, **C)** Mean larval mass (g) and **D)** Brood mass (g). Further to this the graph shows how individuals within separate populations supplied with a different size carcass varied in their plastic response, on the left individuals were given a large carcass only and on the right-hand side individuals were given only a small carcass.

2.5 Discussion

Often it is accepted that competition negatively effects reproductive performance, largely because energetic resources are finite and consequently energy diverted for competition comes at a cost to reproductive investment (Fitzpatrick *et al.,* 1995; Clutton-Brock, 2009). However, this adjustment in reproductive expression has instead also been argued to be an adaptive response (Fox & Mousseau, 1998). Deciphering between the two opposing views is of importance to understand how selection works on social and sexual traits.

The results of this study vary depending on the trait measured. The change in post-natal parental investment in relation to the social environmental change follows more closely with the costly competitive hypothesis, as we observed a significant decline in maternal care in a competitive environment. On the other hand, females adjusted the number of offspring within a brood to the social environment, by reducing the number of larvae in a competitive environment meaning larvae were consequently heavier. This result is therefore more consistent with the adaptive response hypothesis as a way to maximise offspring size in a harsh environment (competitive environment), and consequently the response could be labelled as a form of adaptive transgenerational plasticity (Fox & Mousseau, 1998). Below the results of this chapter are discussed in further detail.

Parental Care and Competition

The balance between parental effort and mating effort (competition) has long been established as an energetic trade-off (van Noordwijk & de Jong, 1986; Roff, 1992; Stearns, 1992). Empirically this hypothesis has been supported numerous times, and similar to this study they have specifically shown that increasing investment into competition causes a decline in parental investment (O'Neal *et al.,* 2008; Cain & Ketterson, 2013; Rosvall *et al.,* 2013).

However, in detail these studies have shown that while certain behaviours like parental brooding/nesting behaviour are reduced, provisioning behaviour is not (O'Neal *et al.,* 2008; Cain & Ketterson, 2013; Rosvall *et al.,* 2013). And in some cases provisioning behaviour increases with increased levels of aggression, which is arguably been described as beneficial for aggressive individuals, and a behavioural strategy adopted by individuals to minimise the effect of aggression on offspring phenotypes (Cain & Ketterson, 2013). This has been developed into an idea that parental and competitive trait evolution is positively correlated (Wolf *et al.,* 1999) but also would support a post-natal investment maternal effect. Yet, in this chapter both direct care (offspring feeding) and indirect care (carcass maintenance and guarding) significantly decline in a competitive environment, suggesting there is no further post-natal reproductive investment to manipulate offspring size at dispersal. This is despite increased provisioning behaviour known to improve offspring growth and survival in burying beetles (Eggert, Reinking, & Müller, 1998). Indicating that investment into female-female competition, to gain access to the reproductive resource, limits the energetic resources available for parental investment and therefore follows the costly competitive hypothesis (Fitzpatrick *et al.,* 1995).

Broadly, these results suggest that competitive and parental traits are negatively correlated in burying beetles. Thus, positive selection acting on one trait would negatively influence the expression of the other trait. For example, it has been shown that selection on aggressive behaviour may affect the evolution in maternal traits (e.g., Sartori *et al.,*. 2015). This is potentially why we see no sexual traits in burying beetles that improve competitive performance, as selection is more favourable towards maternal traits because they are more beneficial across multiple environments compared to competitive phenotypes, thus potentially constraining the evolution of competitive traits.

As well as investigating population level responses, this study also analysed individuals. For all parental traits, female ID did not explain significant amounts of residual variation found within our dataset, suggesting female burying beetles do not vary in the level parental care provided. Yet, as shown in figure 5A both elevation and slope vary highly between individuals. One explanation for these findings is that because the environmental context influences the level parental behaviour provided (Table 1) the same individual is likely to be inconsistent in the amount of parental care provided across environments. Furthermore, the effect between individuals will vary because of the extent of variability within the environment that each individual experienced (e.g. the level of competition experienced). Thus, there will be no pattern in the response to environmental change at an individual level, as shown in figure 5A by the direction and slope of the reaction norms. Broadly, the implications of this result could suggest that the quality of parental care provided in burying beetles and the variation found between individuals seems to depend largely on the past and present environmental conditions that individuals experience, rather than an individual's genetic architecture. Selection favouring further complex levels of parental care in burying beetles are therefore possibly restricted by how the environmental variation individuals experience, relates to changes in the level of energetic investment required to provide parental care.

Reproductive Investment and Competition

Burying beetles are known to plastically adjust their brood size in relation to the environment (Creighton, 2005). Furthermore, it is suggested burying beetles actively manipulate brood size with female density, such that they follow optimal game theory and produce less but larger offspring in higher density environments (Creighton, 2005). The results in this chapter would agree with this result, but instead focused on how females responded after direct competition for the reproductive resource rather than female density.

This chapter therefore followed predictions made by the adaptive response hypothesis (Fox & Mousseau, 1998; Mousseau & Fox, 1998) as well as the conclusions made in similar studies on other taxa (Allen *et al.,* 2008; Bentz *et al.,* 2013; Inzani *et al.,* 2016), females reduced the number of offspring in a brood and mean larval mass increased in a female-female competitive environment. The primary reason it is considered adaptive is because offspring fitness is associated with offspring size, especially in unfavourable environmental conditions (Allen *et al.,* 2008). Consequently, adjusting offspring traits in relation to the environmental conditions can be beneficial. For example, in tree swallows (*Tachycineta bicolor*) females can maximise both their reproductive success and that of the offspring by increasing the amount of yolk testosterone in nestlings in high density environments, as it increases hatchling growth and thus improves the offspring's competitive ability (Bentz *et al.,* 2013). This is a similar scenario in burying beetles, where producing larger offspring in a competitive environment has fitness benefits for offspring and parents. This is because larger offspring usually become dominant individuals and will monopolise reproduction when carcasses are contested for, thus the maternal response to maximise larval size in a competitive environment leads to a greater reproductive success if competition for carcasses remains constant (Müller *et al.,* 1990; Eggert *et al.,* 2008). Consequently, this result would broadly suggest that adaptive maternal responses or transgenerational plasticity to the competitive environment in female burying beetles will be positively selected upon and evolve.

However, careful consideration should be made about the above conclusion and within similar studies. Offspring number and offspring size represents a well acknowledged trade-off in life-history theory, which illustrates that investment into quality comes at a cost to quantity (Lack, 1947; Roff, 1992; Stearns, 1992). The increase in mean larval mass may therefore be an indirect affect rather than a direct response to the social/competitive environmental change. For example, experiencing competition may reduce an individual's brood size because of energetic constraints imposed by investment into competition (van Noordwijk & de Jong, 1986; Roff, 1992; Stearns, 1992), and females are then adapting to brood size following a size-number trade-off (Smith & Fretwell, 1974), and thus not directly responding to the competitive environment but achieving the same result, a smaller brood size but larger offspring in a competitive environment. Separating whether the observed response is an indirect effect or a plastic response or whether they are considered the same thing remains a challenge in future research if we are to understand further the importance of plasticity and its role in social evolution.

The results on the individual level analyses on reproductive traits in this chapter would however direct towards supporting the adaptive response hypothesis. The results demonstrated that there was little variation between females in both their elevation (female ID) and slope (IxE) for both number of offspring and mean larval mass (g). Yet, if the response was seen as an indirect effect, it would be expected that there would be variation between individuals (female ID, IxE), because of the variation in competitive investment (e.g. the level of competition) affecting the energetic resources available for reproductive investment (number of offspring). While I also expected to find significant levels of individual variation in the adaptive hypothesis, if the response is adaptive as theory suggests (Fox & Mousseau, 1998; Mousseau & Fox, 1998), I would also expect that selection would act on the variation between individuals producing an optimal response. And thus, it is a possibility that there is little variation found within this study because of past selection pressures acting on the response.

Unlike the above mentioned reproductive traits, brood mass significantly varied between individuals (female ID) and there was also variation in how they responded to environmental change (IxE). The primary reason why I expect this doesn't follow the pattern of the other reproductive traits is because of other factors influencing the outcome. The quality and level of parental care, offspring begging and competition amongst offspring are all variables likely to have increased the variability found amongst individuals in brood mass at dispersal.

Resource Quality and the Response to Competition

Resource quality had no effect on the plastic response to social environmental change as predicted if the data followed an adaptive response hypothesis. However, we found carcass size effected the number of offspring produced as previously illustrated, with more offspring produced on a larger carcass (Creighton *et al.,* 2009). Additionally, carcass size affected the variation in plasticity and phenotypic expression amongst individuals, with individuals varying greater on larger carcasses. This is most likely because on larger carcasses differences in female quality are exaggerated as greater resources are available but only high quality individuals can benefit from this.

Conclusion

This chapter showed that parents do not adjust parental behaviour to follow the predicted adaptive response. Instead the results showed that competition has costs, by illustrating a decline in parental investment in a competitive environment. However, our main finding is that females are plastically responding to female-female competition by increasing larval mass and reducing offspring number in a brood to maximise their and their offspring's future reproductive success (Fox & Mousseau, 1998; Mousseau & Fox, 1998). The results in this chapter consequently indicate that maternal effects/transgenerational plasticity to the social environment is adaptive.

However as mentioned, caution should be made around this conclusion. Although in this study the results are interpreted as an adaptive response, future efforts should try and further disentangle whether a change in phenotypic expression is because of how the environment affects phenotypic expression based upon the energetic resources or whether the individual is directly manipulating a trait to the environmental que they are exposed to.

Furthermore, this chapter hasn't quantified all the costs and benefits of such a response, so while this chapter found that on average females respond adaptively, this chapter also found that there was a cost to the response, as the average brood mass declines in a competitive environment, despite the same amount of body mass invested into reproduction (Appendix, S1). Future research should therefore also consider other benefits and costs of the plastic response to assess its true benefits, such as whether those that don't respond to the competitive environment suffer greater reproductive costs.

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Supplementary Data

Supplementary data 1 (S1)

Female body mass investment and social environment

Supplementary table 1, Mixed model analyses testing the effect of social environment change on female body mass investment. Significant terms of the fixed effects were obtained using likelihood ratio test following stepwise removal of terms.

Female Body Mass Investment (g)		
Fixed effects	**$\chi 2_1$**	**P value**
Social environment*carcass size	0.039	0.843
Social environment	0.036	0.849
Carcass size	1.591	0.207
Order	0.78	0.377
Beetle size	2.138	0.143

Chapter 3 – Age-dependant Plasticity

3.1 Abstract

Why plasticity differs between individuals and how this influences selection on plasticity has recently been discussed in literature, in order to further understand how plasticity may evolve. Age is a state variable known to influence major life-history traits but few have looked at how it may influence plasticity. Recent theoretical work has discussed such a topic, and it is expected that the optimal response in young individuals is to be responsive, while in old individuals it is beneficial to be non-responsive. However, few studies have empirically analysed such predictions. The main aim of this chapter was therefore to look at how age affected reproductive investment decisions to a change in breeding resource quality using burying beetles. The chapter also aimed to establish the extent of variability in response amongst individuals, broadly to further understand how selection may be acting on plasticity. At a population level the results to a certain extent followed the theoretical predictions; old individuals were non-responsive and young individuals were responsive, however, this pattern was dependant on other environment variables. The results also demonstrated that individuals substantially differed in their plastic response, and more so in old individuals. This suggests that old individuals are plastic and that as individual's age variation in state becomes more pronounced, meaning there is more among individual variation. In the chapter, it is discussed how these results may have an impact on how selection is acting on phenotypic plasticity.

3.2 Introduction

Phenotypic plasticity is the ability of a genotype to express multiple phenotypes in response to environmental change (Schlichting & Pigliucci, 1998; Pigliucci, 2001). If a plastic genotype results in increased survival and reproductive success across the environmental gradient, than a fixed genotype, it would be intuitive to think that natural selection would act on plasticity (Scheiner, 1993; Pigliucci, 2005). Numerous theoretical models have analysed the conditions that favour the evolution of phenotypic plasticity (Houston & McNamara, 1992; Via *et al.,* 1995; Schlichting & Pigliucci, 1998; Pigliucci, 2001). One crucial assumption in these models is that there are sufficient levels of variation in plasticity for selection to act upon. Thus, to fully understand how natural selection acts on plasticity we need to analyse both the genetic variation (GXE) of plasticity (Via & Lande, 1985; Scheiner & Lyman, 1989; Gomulkiewicz & Kirkpatrick, 1992), but also how plasticity varies between and within individuals (IxE) (Dingemanse, Kazem, Réale, & Wright, 2010; Dingemanse & Wolf, 2013; Nussey, Wilson, & Brommer, 2007).

Exploring individual level variation (IxE) in plasticity, until recently has been overlooked (Nussey *et al*., 2007). Individual variation in phenotypic expression is determined by both genetic and non-genetic mechanisms. Environmental factors that drive IxE are potentially adaptive, influencing the rate and trajectory of evolutionary change, by altering the selective pressures acting on plastic traits (Alonzo, 2015; Dingemanse & Wolf, 2013; Nussey *et al*., 2007; Westneat, Wright, & Dingemanse, 2014). What ecological forces that drive both between and within individual variation in plasticity and whether this variation is adaptive has consequently become a fundamental question in evolutionary research (Alonzo, 2015; Dingemanse & Wolf, 2013; Nussey *et al*., 2007; Stamps, 2015; Westneat *et al*., 2014).Using the reaction norm framework, empirical studies have begun investigating how the environment may determine patterns of adaptive individual variation in plasticity (Biro, Beckmann, & Stamps, 2010; Mathot *et al*., 2011; Nussey, Clutton-Brock, Elston, Albon, & Kruuk, 2005; Westneat, Hatch, Wetzel, & Ensminger, 2011). One variable yet to be explicitly analysed is how plasticity changes over an individual's lifetime and whether plasticity is age-dependant (Fischer *et al.,* 2014).

An individual’s age shapes important life-history decisions. For instance, it determines an individual’s current reproductive strategy (e.g. terminal investment or reproductive restraint), as the benefits and costs of current vs future reproductive investment are age-dependant (Williams, 1966; Clutton-Brock, 1984; Roff, 1992; Stearns, 1992; McNamara & Houston, 1996). Simply this means that an adaptive reproductive strategy will vary over an individual’s lifetime (Roff, 1992; Stearns, 1992; McNamara & Houston, 1996). Recently, it has been suggested that plasticity is also age-dependant and differences in IxE are the result of an age related adaptive response (Fischer *et al.,* 2014). The reason for this prediction is that the costs and benefits of plasticity are context-dependant (Houston & McNamara, 1992, 1999; Clark & Mangel, 2000; Fischer *et al.,* 2014). An individual’s optimal level of plasticity will therefore vary with age if the benefit and costs of plasticity change, as they are theoretically predicted to do so (Fischer *et al.,* 2014). Specifically, it is suggested that maintaining plasticity when young and have a high reproductive potential will be adaptive, as individuals can reduce the chance of a costly phenotypic- environment mismatch. Additionally, they suggest plasticity will increasingly be disfavoured over an individual’s lifetime because the potential beneficial effects of a plastic response are reduced, as the chance of future reproduction is low (Fischer *et al.,* 2014). Consequently, it is expected that overtime individuals will become less responsive to environmental change and that it is adaptive to do so (Fischer *et al.,* 2014). While there is considerable empirical support for the theoretical predictions in developmental and sexually selected traits (e.g., Atwell & Wagner, 2014; Scott, McAbee, Eastman, & Ravosa, 2014), relatively little work has looked at behavioural and reproductive traits.

Parental care and reproductive traits provide a promising basis in which to explore age-dependant plasticity. This is because the costs and benefits of an individual’s reproductive decision vary with respect to the environment, thus favouring phenotypic plasticity in heterogeneous environments (Clutton-Brock, 1991; Roff, 1992; Stearns, 1992). The expression of parental care and the level of reproductive investment has therefore often been shown to be age-dependant (e.g., Clark, Moghaddas, & Galef, 2002; Clutton-Brock, 1984). However, to our knowledge no paper has yet analysed whether plasticity in these traits is age dependant and the extent of variability in phenotypic plasticity between individuals (IxE). This seems fundamentally important as understanding how multiple environmental pressures interact and how this influences reproductive traits is likely to have implications on selection. This chapter therefore attempts to address this current gap in our understanding by exploring whether plasticity in parental care and reproductive investment is age-dependant, using burying beetles (*Nicrophorus vespilloides*) as our model system. Furthermore, this study looked to understand the patterns in individual responses and how this may affect reproductive performance.

As in all *Nicrophorus* species, *N.vespilloides* breed exclusively on small vertebrate carcasses (~4-30g)(Scott, 1998). The size of the carcass often reflects eventual brood size, as parents adaptively adjust the number of offspring (Creighton, 2005; Creighton, Heflin, & Belk, 2009; Smith, Creighton, & Belk, 2015; Trumbo & Fernandez, 1995), by manipulating the number of eggs laid or by filial cannibalism (Bartlett, 1987; Müller & Eggert, 1990). As a result an increased carcass size has an increased brood size (Creighton, 2005; Trumbo & Fernandez, 1995). Additionally, it has been demonstrated that when individuals breed multiple times on different carcass sizes, the order in which they are manipulated affects their reproductive strategy (Billman *et al.,* 2014). Thus, prior experience of carcass size affects allocation to current reproduction (Billman *et al.,* 2014). Furthermore, studies on burying beetles have illustrated that reproductive investment strategies vary over an individual's lifetime and generally we see a decline in fecundity with age (Cotter, Ward, & Kilner, 2011; Creighton *et al*., 2009). *N.vespilloides* also provide extensive pre- and post-natal care. Both sexes provide multiple forms of care including direct care (feeding offspring) and indirect care (the maintenance and defence of breeding resources) which increases offspring fitness and survival (Eggert, Reinking, & Müller, 1998; Scott, 1998). The amount and duration of parental care is highly variable due to variation in the environment (Smiseth, 2004; Smiseth & Moore, 2002; Smiseth, Dawson, Varley, & Moore, 2005). This includes female age which is shown to significantly influence maternal behaviour. As a result, older individuals spend more time in the carcass with offspring, consequently reducing the decline in fecundity they experience with age (Lock *et al.,* 2007).

Despite the established independent effects of age and carcass size on reproductive investment strategies and parental care in burying beetles, little is known about how individual age and resource quality may interact. This seems biologically important, as opportunities for individuals to breed are stochastic. Experiencing variation in resource quality in relation to age would therefore be expected which may have implications on their life-history strategies. For example, an old beetle on a small carcass may respond differently to a young beetle that has future breeding opportunities. By manipulating female age and carcass size in burying beetles is it therefore possible to determine whether plasticity is age-dependant.

The first aim of this study was therefore to establish the population level and individual level plastic response in parental behaviour and the number of offspring produced in response to environmental change (carcass size) of females at different ages. In this chapter, it was predicted that a female's response to carcass size will be age-dependant, specifically, expecting that older individuals will be less responsive and young individuals will respond to the change. The reason for this prediction is that reducing/or restraining reproductive investment at an older age will provide no benefit as the chance of future reproduction is low. The number of larvae and the time spent providing parental care will consequently remain the same irrespective of carcass size. However, we expect this pattern of age-dependant plasticity to be dependent on order effects (the order in which they received a large and small carcass). This is because we expect younger individuals to restrain reproduction in their first breeding attempt, while older individuals show an increased investment in their first attempt. In the paper, I also wanted to establish individual patterns in plasticity because if there is evidence of IxE natural selection can act on the variation. We expected evidence of IxE and that a greater variation in plasticity will occur in the older population due to a greater variation in female quality.

3.3 Methodology

General methodology and maintenance

A stock population of *N. vespilloides* were caught from Devichoys Wood, Cornwall, UK (N50^{o}11'47"E5^{o}7'23") during August 2015, comprising of 50 males and 50 females. They were subsequently randomly paired and placed into individual breeding boxes (17x12x6cm) filled with damp soil and a mouse carcass (15-25g; Livefoods Direct, Sheffield). Once larvae dispersed from the carcass they were then transferred into individual rearing containers (7x7x4cm) and placed in incubators at 21^{o}C (±2^{o}C) with a 16L:8D hour cycle. Following pupation, they were checked daily to determine date of eclosion, before being fed two decapitated mealworms (*Tenebrio*) twice weekly until they were sexually mature at 14 days of age. This cycle was then repeated till the fourth generation, allowing us to sufficiently create three breeding blocks to maximise our sample size without logistical problems. Experimental stock were then treated as above, apart from the older age cohort being fed for an extra week. All individuals were virgin and socially naïve before beginning the experiment irrelevant of the treatment individuals were placed in. At 10 days old beetle pronotum width was measured three times and averaged using digital calipers (to 0.1mm).

Experimental design

To analyse how the interaction between reproductive investment and carcass size alters with age, we used a 2x2 factorial manipulating female age and carcass size (mean±SD). We also used a repeated measures design, where each individual breed consecutively on a large (24.1±1.46g) and small carcass (12.3±1.12g). Our total sample size was N=163 individuals.

As in other studies analysing age effects on reproductive investment in *N. vespilloides*, females were assigned to one of two age treatments post-eclosion (Benowitz *et al.,* 2013). On the day of first experimental breeding it meant young individuals were 10-12 days old, while older individuals were 23-25 days old. This age was chosen to maximise the age difference between treatments without substantial mortality, which occurs from the fourth to fifth weeks of age (Benowitz *et al.,* 2013). However, old individuals were younger than previous studies because each individual had two breeding bouts with 48 hours before the first and second breeding attempt. Consequently, in the second round young individuals were between 18-19 days old and older individuals were 31-33 days of age. Within each age treatment individuals were also randomly assigned to a large (24.1±1.46g) or small (12.3±1.12g) carcass for their first breeding bout. By alternating the start environment within each group, it allowed us to control for order effects that may occur between individuals due to variation in the environment they experience at different ages but also allowed us to investigate other potential patterns of investment. During the second breeding attempt individuals were then exposed to the opposing carcass, so that they experienced both environments. Ultimately, this repeated measures design allowed us to look at individual level variability in their response to carcass size across different age cohorts.

Prior to breeding, females were pre-mated to randomly selected males to minimise any behavioural effects due to their presence. After 48 hours which allowed sufficient time for fertilisation (Müller & Eggert, 1989), males were then removed and females were provided with a carcass. Throughout the experiment, we then measured parental effort during a key phase of larval growth and survival, as discussed below. At larval dispersal, offspring number and brood mass were measured as a proxy for fitness (Rauter & Moore, 2004a) and to analyse individual adjustments in brood size to carcass size, as previous evidence suggests (Trumbo & Fernandez, 1995; Creighton, 2005). Additionally, we also measured individual mass as a proxy for their quality. This was done before and after both breeding attempts, allowing us to analyse how individuals may alter their investment and whether they conserve energy at a younger age.

Parental Care observations

Parental observations took place around 24, 30 and 40 hours after first larval hatching, a key phase in larval growth and survival because it is at its peak of intensity (Smiseth, Darwell, & Moore, 2003). During these four periods, we used instantaneous scan sampling (Martin and Bateson, 1993) recording individual behaviour every 1 minute for 30 minutes, as previous papers have suggested (Smiseth & Moore, 2004). However, before any observations individuals were acclimatised to the room for a period of 30 minutes (Smiseth *et al*., 2005). To minimise any disturbance on behaviour caused by the observer, all observations were recorded using infrared surveillance cameras (N08CX night vision CCTV camera). By placing the camera into a nicrocosm (400mm length of black PVC-U Ø 110mm) within the breeding box, we used motion detection software (AverMedia NV6240 Express, dvr version 7.7.0.0007; www.avermedia-dvrs.com) to record parental behaviour (Further details see, Hopwood *et al*., 2013). Parental behaviour was recorded as either direct care (regurgitation of carrion to offspring, direct mouth to mouth contact) or indirect care (carcass processing and maintenance, moving the carcass and adding antimicrobial secretions)(Scott, 1998; Smiseth & Moore, 2004). All observations were at 21^{o}C (±2^{o}C).

Statistical analysis

Population responses- In order to analyse whether age influences the response to carcass size, we performed linear mixed models in R version 3.0.2 (R Core Team 2015) using package lme4 (Bates *et al.,* 2015). In these models, we included carcass size, age and order as fixed effects as well as all interactions between them. We also included beetle size as a covariate within the model and female ID was included as a random effect. To achieve the minimum adequate model we used likelihood ratio test to compare models, starting with interaction terms, until only significant terms remained (P<0.05). The package lmertest (Kuznetsova *et al.,* 2015) was then used to obtain significance tests of F values for the minimum adequate model as well as to test the significance of the random effect structure. This method was used for the following response variables; number of offspring, mean larval mass, brood mass and decline in body mass due to reproduction. To assess female parental care, we used a general linear model with same fixed effect structure but the model had a binomial error structure. The model had a random effect structure with female ID (1|ID) and an observation level effect (1|observation) a method used to account for overdispersion (Harrison, 2014). Significant fixed effect terms were achieved using the likelihood ratio test starting with interaction terms.

Individual responses- To assess whether females on average varied significantly in their trait expression we tested the random effect, female ID, for all measurements. Having first removed non-significant from our main model, we then used a chi-square likelihood ratio test with one degree of freedom to test this model against the same model with female ID removed. We then assessed the significance of female ID separately for different age groups by subsetting the data and repeating the above process. This was to analyse differences in individuals within a population caused by age effects.

Using the package ASReml (Gilmour *et al.,* 2009) we then tested whether individuals varied in their plastic response to resource quality (IxE). Specifically, we investigated how phenotypic variance (conditional on the fixed effects) is distributed across our two traits in separate environments and within each age category. This was achieved by a bivariate analysis of a mixed model where each row represents an individual and contains the response variable on a small and large carcass. To determine statistical significance of variance terms I compared a set of nested models with different (co)variance structures using the likelihood ratio test (Visscher, 2006). The first model included no covariance but also the variance was constrained to be the same in both a large and a small carcass. This first model was then compared to the second model allowing different variance estimates for both a large and small carcass. A significant result would indicate individual plasticity assuming measurement error to be equal across environments, this is because unequal variances across environments means individuals must vary in how they respond to a change in the environment. This second model was then compared against an unstructured covariance model, meaning traits could co-vary as well as allowing separate variance terms for large and small carcass. This result indicated whether there is significant individual covariance across environments; the correlation across environments can then be calculated using $COV(_{E1,E1})/\surd(V_{E1}*V_{E2})$. The correlation term indicates the strength and sign of the covariance – for example, a strong positive term indicates that generally performance is parallel across environments (such that an individual with high performance in one environment perform well in the other). Bivariate models were run separately for each age treatment by sub-setting the data to again analyse differences in individuals in a population to age effects. Additionally, the effect of order was mean centred to account for variation among age groups due to the order effect.

3.4 Results

Parental behaviour

Population plasticity- The total level of parental care provided by females depended upon the three way interaction between carcass size, age and order of the environment ($\chi 2_1$=6.256, P=0.012, table 1): On average the older age group responded similarly to carcass size change, regardless of the order in which they experienced carcass sizes (figure 1a). On the other hand, the plastic response to carcass size in young females depended on the order of the environment, demonstrated by the direction of slope differing between treatments (figure 1a). Furthermore, older individuals on average spent more time providing care (table 1). Beetle size did not influence the level of parental care provided ($\chi 2_1$=0.087, P=0.434).

Individual plasticity- Adding female ID as a random effect significantly improved the fit of model ($\chi 2_1$=6.806, P=0.009) and explained 22.21% of the variation in parental behaviour. The amount of parental care provided therefore differed among individuals. However, when sub-setting the data into age cohorts, we found that younger individuals did not differ in the level of parental care provided ($\chi 2_1$=1.433, P=0.231), but older individuals did ($\chi 2_1$=5.526, P=0.018) with female ID explaining 38.3% of the variation. The structure of model limited our ability to assess individual plasticity (IxE) and find the potential patterns in their response, however, figure 1b graphically illustrates the result demonstrating variation in individual responses.

Table 1, Minimum adequate model of a general linear mixed model analysing how females plastically adjust parental care in relation to carcass size.

Response	Fixed effects	Effect size±SE	Z	P value
Total level of parental care	Intercept(old,large,L-S)	-0.917±0.666		
	Age x Carcass size x Order	-4.349±1.729	-2.515	**P=0.011**
	Carcass size x Age	1.429±1.223	1.169	P=0.242
	Carcass size x Order	0.445±1.18	0.377	P=0.706
	Age x Order	0.999±1.365	0.732	P=0.464
	Carcass size	1.369±0.848	1.612	P=0.107
	Age	-2.199±0.975	-2.255	**P=0.024**
	Order	0.924±0.931	0.994	P=0.32

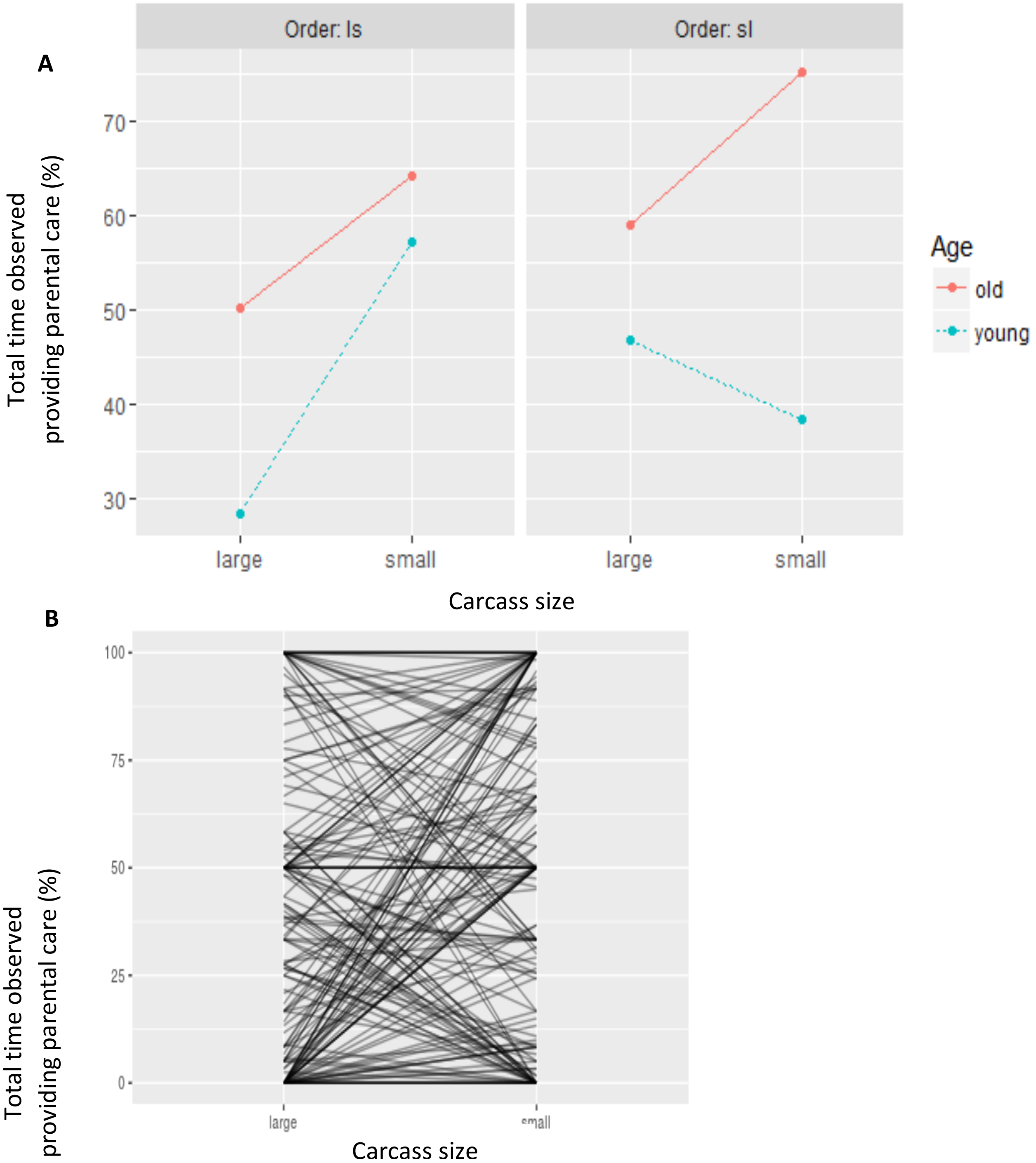

Figure 1, demonstrates the population and individual level responses in parental behaviour (average time spent providing care (%)) in relation to the environmental variables age, carcass size, order and the interaction between them. In figure **1a** it shows the population level response to the three-way interaction. Age populations are represented by the lines, old (red, filled line) and young (blue, dotted line). Order effect is demonstrated across graphs, where sl represents individuals breeding on a small then large carcass and ls the opposing pattern. Carcass size is on the x axis. The graph shows an interaction, as shown by the variance in the gradient of the line between the populations. Figure **1b** shows the individual level responses in parental behaviour to carcass size, it highlights the extent of variability found between individuals in their response to environmental change, as shown by the large variation in the direction and gradient of the slopes in the graph.

Number of offspring

Population plasticity- A female's plastic response to carcass size was found to be dependent on age and the order effect ($\chi2_1$=22.07, P<0.001, table 2). It was found that older females were less responsive to environmental change than younger individuals (table 2, figure 2). However, the degree of response was dependent on the order effect which either emphasised or reduced the effects of age on female plasticity (figure 2). There was a significant main effect of carcass size, with 3.65±1.21(SE) more larvae on a larger carcasses. Beetle size also significantly influenced the number of offspring produced, with larger individuals producing more offspring ($\chi2_1$=22.07, P<0.001, table 2).

Table 2, Minimum adequate model of a linear mixed model analysing how females plastically adjust the number of offspring within a brood in relation to carcass size.

Response	Fixed effects	Effect size±SE	F	df	P
Number of Offspring	Intercept(old,large,L-S)	3.368±7.487			
	Age x Carcass size x Order	-11.778±2.423	23.631	1,163	**P<0.001**
	Carcass size x Age	-1.623±1.726	38.457	1,163	**P<0.001**
	Carcass size x Order	3.48±1.666	3.951	1,163	**P=0.048**
	Age x Order	5.636±2.557	0.013	1,163	**P=0.013**
	Carcass size	-3.658±1.205	87.763	1,163	**P<0.001**
	Age	0.047±1.819	0.637	1,163	P=0.426
	Order	-0.721±1.746	0.633	1,163	P=0.427
	Beetle size	4.636±1.629	8.095	1,163	**P=0.005**

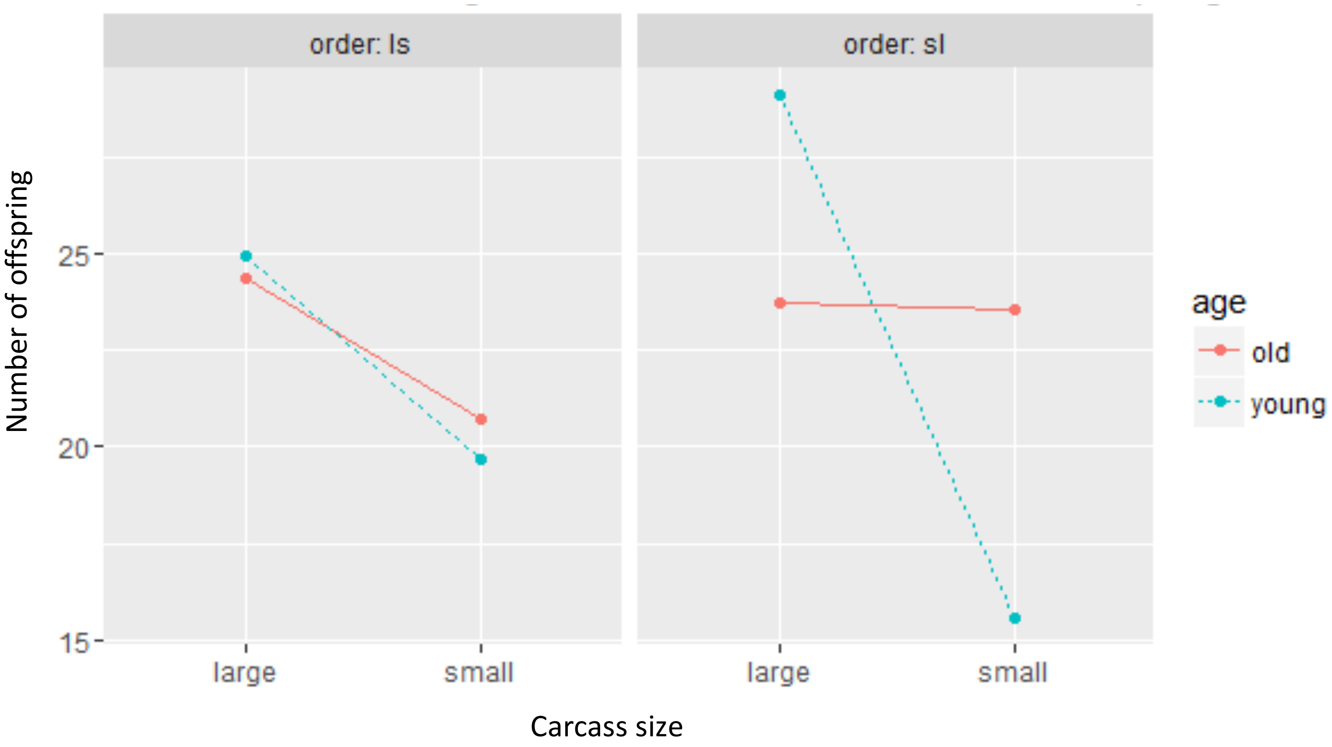

Figure 2, illustrates the population level response in the average number of offspring produced in relation to the interaction between the environmental variables age, carcass size and order. Age populations are represented by the lines, old (red, filled line) and young (blue, dotted line). Order effect is demonstrated across graphs, where sl represents individuals breeding on a small then large carcass and ls the opposing pattern. Carcass size is on the x axis. The figure indicates a strong three-way interaction between the environmental variables on the average number of offspring produced in a population. It is shown by the variance in gradient in the reaction norms within the graph. Furthermore, the figure shows that order effect had a large influence on whether old and young individuals respond differently to carcass size.

Individual level plasticity- Including female ID as a random effect significantly improved the fit of the model (table 3), the number of offspring produced therefore significantly differs amongst individuals and explained 54.47% of the variation in the number of offspring produced. Furthermore, our data suggests ID explains similar amounts of variation within each age group (old= 56.6%, young=51%).

In our study, I also find that the plastic response to carcass size varies amongst individuals (IxE) in both age cohorts (table 3), demonstrated by the variance in the number of larvae differing across environments as well as the significance in covariance. It is graphically illustrated in figure 3. These results also highlight that the number of offspring produced are more variable on larger carcasses and that older individuals were generally more variable in their phenotypic expression (table 3, figure 3). Lastly, a relatively high correlation value in both age cohorts meant, generally higher quality individuals produced more offspring in both environments.

Table 3, demonstrates the significance of the random effect Female ID in the linear mixed model using likelihood ratio test. Secondly, it shows the bivariate analysis of a mixed model to interpret whether plasticity varies between individuals, in which I used the likelihood ratio test to test if models significantly differed.

Model		**Individual plasticity (IxE)**				
Response- Number of offspring	**Female ID**	**Variance (Small) VS**	**Variance (Large) VL**	**Vs=VL**	**Covariance**	**R±SE**
Full population	$\chi2_1=56.4$, $P<0.001$	-	-	-	-	-
Old cohort	$\chi2_1=32.9$, $P<0.001$	56.51±8.77	99.37±15.43	$\chi2_1=3.568$, $P<0.001$	$\chi2_1=24.33$, $P<0.001$	0.527±0.083
Young cohort	$\chi2_1=23$, $P<0.001$	44.02±7.24	68.26±11.19	$\chi2_1=6.377$, $P<0.001$	$\chi2_1=36.24$, $P<0.001$	0.593±0.071

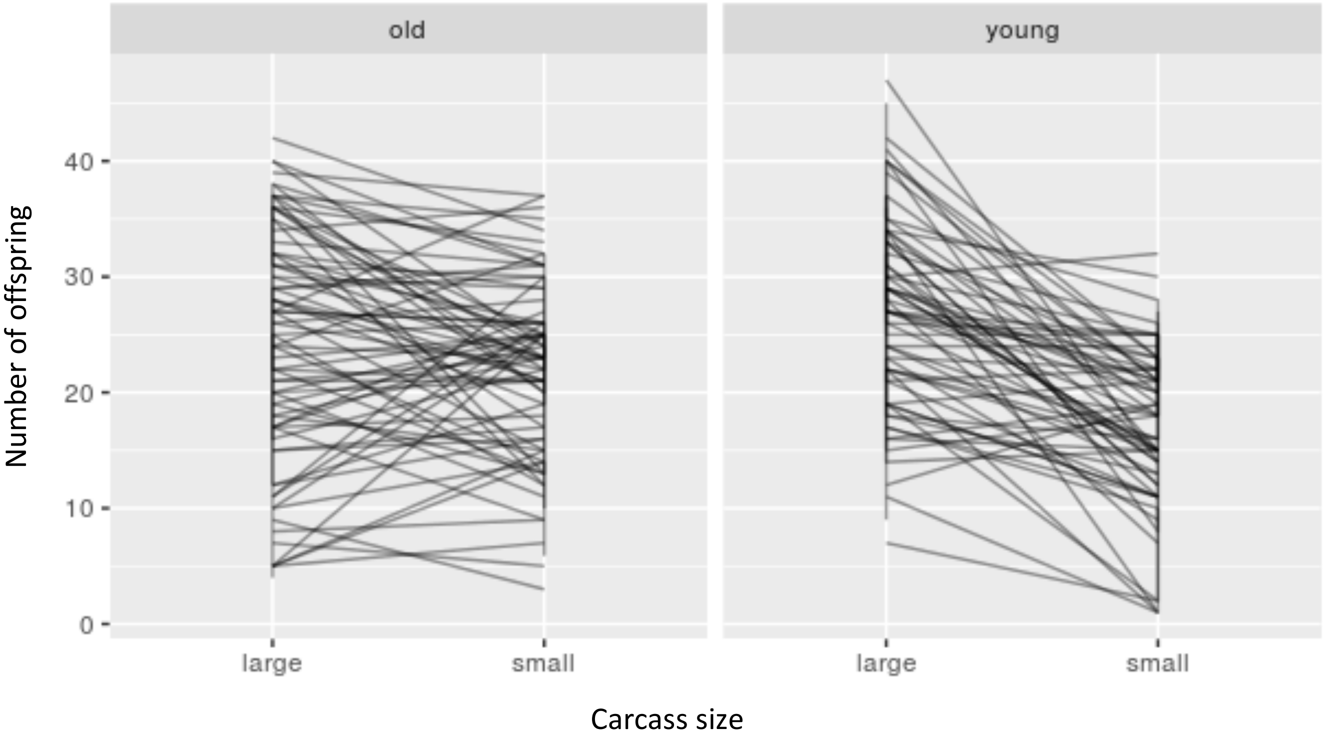

Figure 3, demonstrates the individual level responses in the number of offspring produced in relation to the environmental change, carcass size. The data has then been further sub-set into age groups, old (right) and young (left) to analyse the effect of age on variation in individual responses. The figure is therefore indicating that because the degree and direction of the slopes are more variable in the old population, the level of variation in plasticity to carcass size is greater in older populations.

Mean Larval Mass

Population effect- The three-way interaction between carcass size, age and order effecting the mean larval mass within a brood was not significant ($\chi2_1=1.336$, $P=0.247$), nor was beetle size ($\chi2_1=3.394$, $P=0.065$) or the age x order interaction ($\chi2_1=0.71$, $P=0.399$). However, there was a significant interaction between carcass size and order ($\chi2_1=20.05$, $P<0.001$, table 4): Individuals did not alter larval size if given a large then small carcass, conversely, those that breed on a small then large carcass produced heavier larvae on the large carcass (figure 4a). There was also a significant interaction between carcass size and age ($\chi2_1=47.35$, $P<0.001$, table 4): Older individuals produced heavier larvae at dispersal on larger carcasses, while young individuals produced larger offspring on small carcasses (figure 4b).

Table 4, Minimum adequate model of a linear mixed model analysing the effect of the plastic response to environmental change on the response variable mean larval mass within a brood

Response	Fixed effects	Effect size±SE	F	df	P
Mean Larval mass of brood	Intercept(old,large,L-S)	0.166±0.004		1,163	
	Carcass size x Age	0.046±0.006	54.942	1,163	**P<0.001**
	Carcass size x Order	-0.028±0.006	21.336	1,163	**P<0.001**
	Carcass size	-0.02±0.005	14.055	1,163	**P<0.001**
	Age	-0.023±0.004	0.041	1,163	P=0.84
	Order	0.015±0.004	0.024	1,163	P=0.87

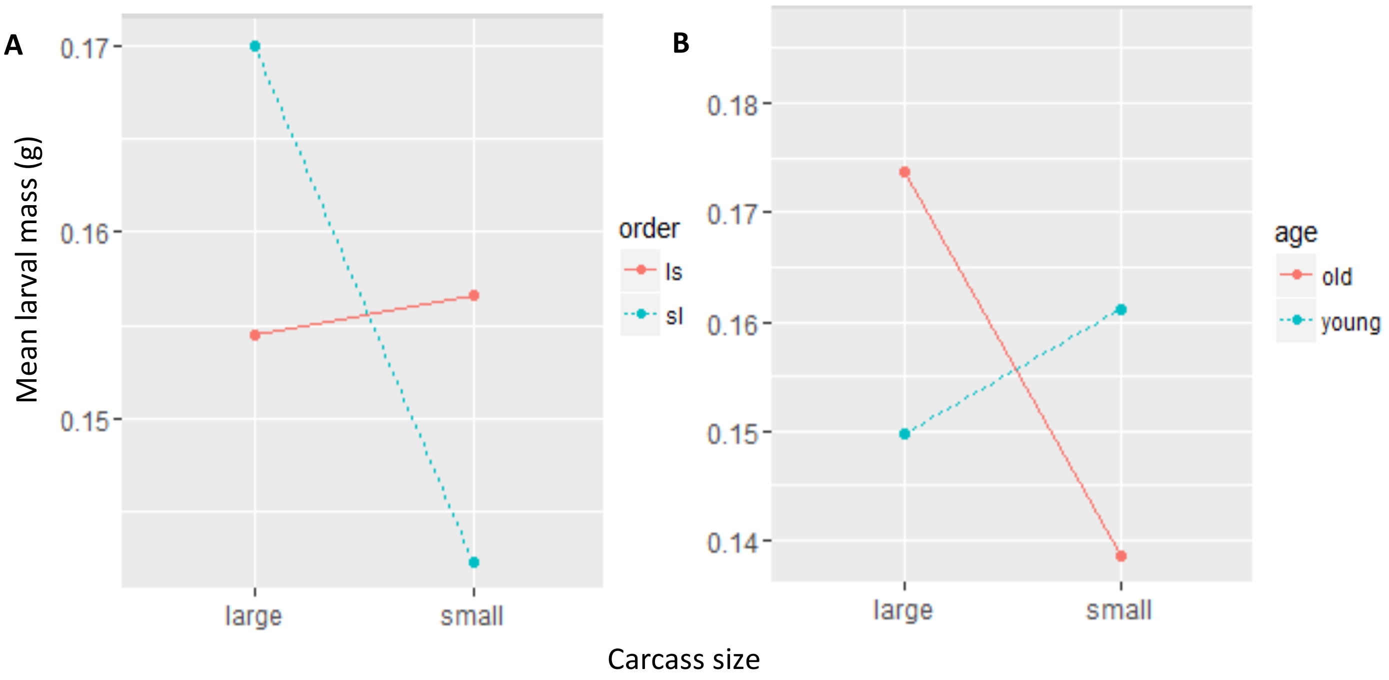

Figure 4, illustrates the population level response in the mean larval mass (g) in relation to the interaction between the environmental variables carcass size and order effect (**figure 4a**) and carcass size and age (**figure 4b**), as the three-way interaction was statistically non-significant. Figure **4a** shows that the order in which they breed on a small and large carcass influences whether they adjust mean larval mass (g), as shown by the almost flat reaction norm against a slopped line. In figure **4b** it also shows that there was an age effect. Young individuals adjusted the mean larval mass (g) in relation to the change in carcass size in the opposing manner to older individuals, as shown by the difference in the direction of slope.

Individual level effect- Female ID was marginally non-significant, however, in the young population alone mean larval mass within the brood varied significantly amongst individuals (table 5) explaining 23.57% of the variance. Our data also shows that the mean larval mass within a brood is more variable in younger individuals (table 5, figure 5). There is also no difference in variance across environments in either age group (table 5, figure 5) but there is a significant covariance in the younger population (table 5). This data is illustrated graphically in figure 5.

Table 5, demonstrates the significance of the random effect, Female ID in the linear mixed model using likelihood ratio test. Secondly, it shows the bivariate analysis of a mixed model to interpret whether plasticity varies between individuals, in which I used the likelihood ratio test to test if models significantly differed.

Model		**Individual effect**				
Response- Mean larval mass	**Female ID**	**Variance (Small) VS**	**Variance (Large) VL**	**Vs=VL**	**Covariance**	**R±SE**
Full population	$\chi2_1$=3.66, P=0.06	-	-	-	-	-
Old cohort	$\chi2_1$=0.158, P=0.7	0.0006± 9.82e-05	0.0008± 1.35e-04	$\chi2_1$=2.13, P=0.072	$\chi2_1$=0.123, P=0.363	0.038±0.109
Young cohort	$\chi2_1$=4.29, P=0.04	0.0010± 1.68e-04	0.0012± 2.02e-04	$\chi2_1$=0.592, P=0.22	$\chi2_1$=4.11, P=0.021	0.231±0.11

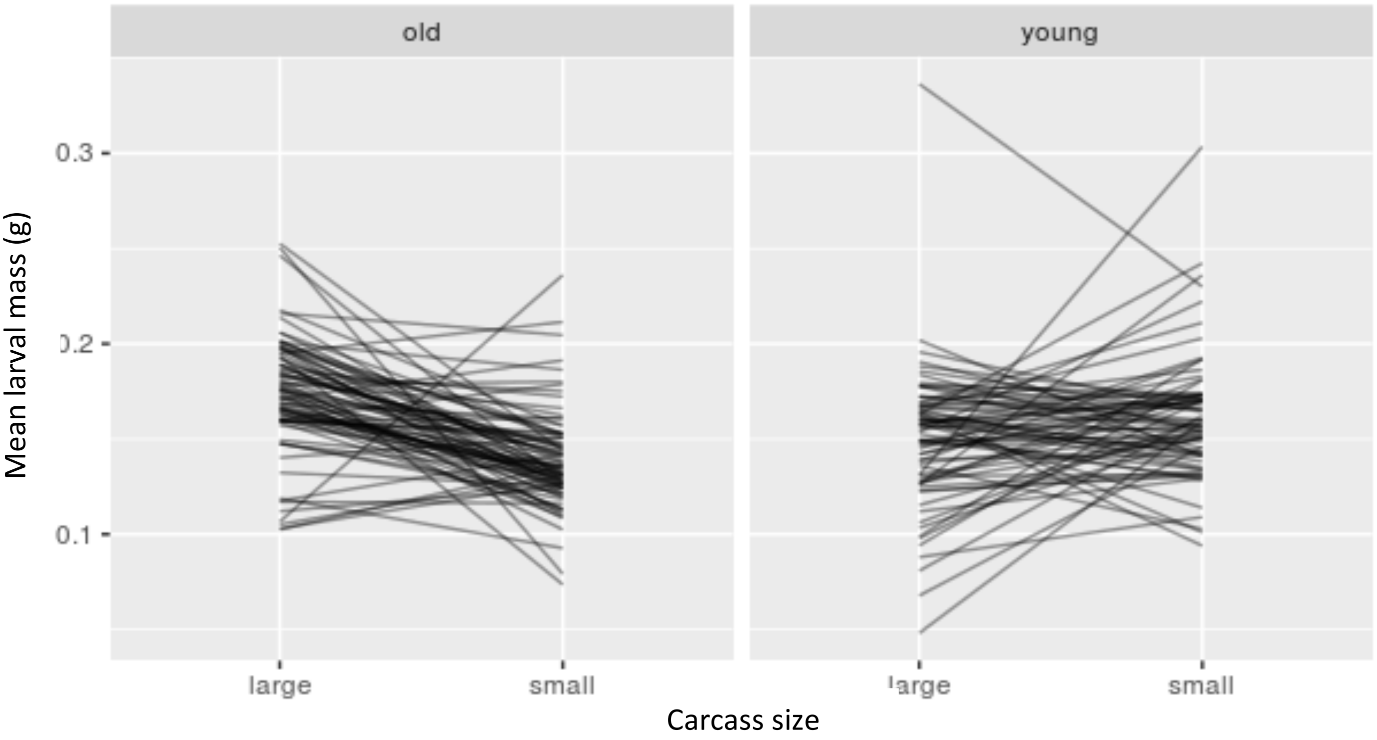

Figure 5, demonstrates the individual level responses in the adjustment in mean larval mass (g) in relation to the environmental change, carcass size. The data has then been further sub-set into age groups, old (right) and young (left) to analyse the effect of age on variation in individual responses. It indicates that there is a general trend in the direction and degree of slope/plastic response between individuals, however the general trend differs depending on the age group.

Brood mass

Population effect- As previously demonstrated larger beetles had a greater brood mass ($\chi2_1$=12.39, P<0.001, table 6). We found the interaction between carcass size, age and order was significant ($\chi2_1$=16.459, P<0.001, table 6). This is graphically shown in figure 6 and demonstrates that the fitness benefit of breeding on larger carcasses relates to an individual's age and whether they breed on a small or large carcass first. This pattern is clearly demonstrated in the younger population while the order effect minimally effects older population.

Table 6, Minimum adequate model of a linear mixed model analysing the effect of the plastic response to environmental change on the response variable brood mass

Response	Fixed effects	Effect size±SE	F	df	P
Brood mass	Intercept(old,large,L-S)	0.0642±1.179			
	Age x Carcass size x Order	-2.044±0.491	17.319	1,163	**P<0.001**
	Carcass size x Age	0.855±0.35	0.46	1,163	P=0.498
	Carcass size x Order	0.077±0.337	14.806	1,163	**P<0.001**
	Age x Order	1.105±0.431	0.055	1,163	P=0.814
	Carcass size	-1.213±0.244	105	1,163	**P<0.001**
	Age	-0.575±0.306	0.361	1,163	P=0.548
	Order	0.121±0.294	1.308	1,163	P=0.254
	Beetle size	0.918±0.256	12.873	1,163	**P<0.001**

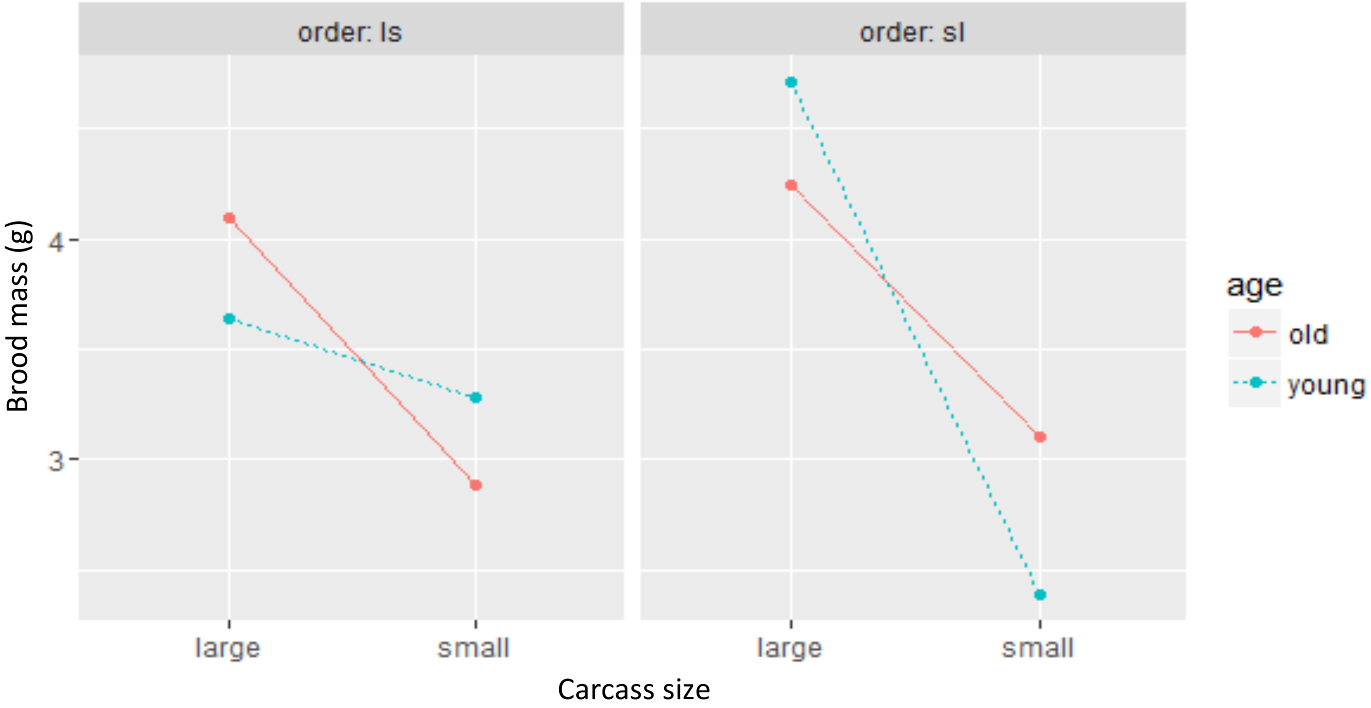

Figure 6, illustrates the population level response in the average brood mass (g)produced in relation to the interaction between the environmental variables age, carcass size and order. Age populations are represented by the lines, old (red, filled line) and young (blue, dotted line). Order effect is demonstrated across graphs, where sl represents individuals breeding on a small then large carcass and ls the opposing pattern. Carcass size is on the x axis. The figure shows that the three environmental variables interact which influence the plastic response to carcass size, as shown by the variance in the gradient of slope within and between the graphs. However, it also shows that the order effect had little effect on the plastic response in old individuals, as the red lines show a similar direction and gradient. Whilst, in the young population (blue lines) order effects had a substantial influence on the change in brood mass (g) across carcass sizes.

Individual level effect- Brood mass significantly varied between individuals (34.2%) (table 7). Furthermore, this was true for both the old (31.6%) and young (37.2%) populations (table 7). For both age cohorts we found a significant difference in the phenotypic variance in the two environments as well as a significant covariance (table 7). The variance in brood mass was greater on a larger carcass (table 7, figure 7).

Table 7, demonstrates the significance of the random effect, Female ID in the linear mixed model using likelihood ratio test. Secondly, it shows the bivariate analysis of a mixed model to interpret whether plasticity varies between individuals, in which I used the likelihood ratio test to test if models significantly differed.

Model		**Individual effect**				
Response- Brood mass	**Female ID**	**Variance (Small) VS**	**Variance (Large) VL**	**Vs=VL**	**Covariance**	**R±SE**
Full population	$\chi 2_1=20.1$, $P<0.001$	-	-	-	-	-
Old cohort	$\chi 2_1=9.12$, $P=0.003$	0.856± 0.132	3.11± 0.482	$\chi 2_1=31.83$, $P<0.001$	$\chi 2_1=13.95$, $P<0.001$	0.393±0.092
Young cohort	$\chi 2_1=11.5$, $P<0.001$	1.056± 0.173	2.668± 0.437	$\chi 2_1=15.39$, $P<0.001$	$\chi 2_1=14.38$, $P<0.001$	0.418±0.095

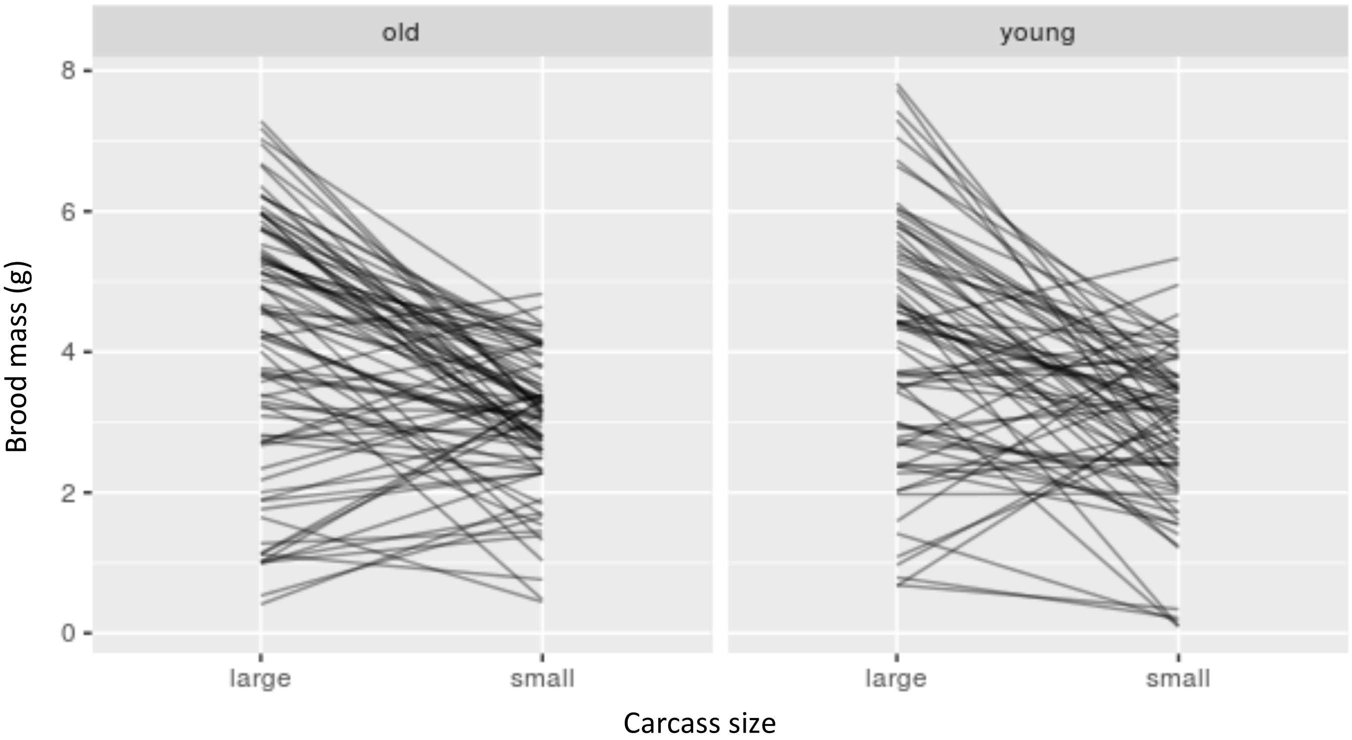

Figure 7, demonstrates the individual level responses in the change in brood mass (g) in relation to the environmental change, carcass size. The data has then been further sub-set into age groups, old (right) and young (left) to analyse the effect of age on variation in individual responses. The figure first illustrates that brood mass (g) between individuals was greater on a large carcass. It also shows that both age populations show a great level of individual variation in plasticity, as shown by the variance in the degree and direction of the slopes in both graphs.

3.5 Discussion

Theoretical research predicts plasticity to decline with age because the life-history strategy influences the optimal level of plasticity by altering the benefits and costs of the plastic response. It is therefore expected that individuals with a greater reproductive potential (young individuals) will be more plastic so as to minimise the cost to reproduction due to environmental change (Fischer *et al.,* 2014). Such age-dependent plasticity could have major implications for the evolution of plasticity, as how selection acts on plasticity would depend on the age structure of a population.

The results in the current study support theory in that plasticity changes with age in both parental care and reproductive investment (number of offspring) traits. However, the direction and the magnitude of these effects differed among traits, depending on the previous and current breeding environment and depending upon whether plasticity was assessed from a population or individual perspective. For instance, how females adjusted their parental behaviour to carcass size depended on their age and their previous breeding environment, showing that both young and old individuals are responsive. A similar three-way interaction was found for how females adjusted the number of offspring to carcass size, and illustrated that at a population level old individuals do not respond, while young individuals do, following Fischer's *et al* (2014) predictions. However, again this observation was dependant on the current and previous breeding environment experienced, such that when they breed on a large and then small carcass, old and young individuals respond almost identically. Additionally, the data demonstrated that how females plastically adjust the number of offspring produced in relation to the environment significantly varied amongst individuals within each age group. These results therefore show that old individuals are more variable in their response, firstly highlighting that old individuals do respond contrary to the predictions made, and secondly that variation in plasticity amongst individuals becomes more pronounced with age.

Lastly, this chapter looked at what the potential implications of the plastic response are on reproductive success. The results demonstrated that old individuals produce larger offspring on a large carcass, while young individuals produce larger offspring on a small carcass, and that generally individuals respond in a similar manner and there is little amongst individual variation (IxE). Interpreting this affect is potentially important to consider as larval mass largely determines body size in burying beetles, a trait associated with fitness (Scott, 1998). However, altering parental and reproductive investment traits had no effect on the overall brood mass on either size of carcass. At a population level this suggests that despite young and old individuals expressing different phenotypes in different environments, it has no effect on their reproductive success. Although, the order of the carcass in which they reproduced on did affect the brood mass, as old individuals performed better in the first breeding attempt and young individuals performed better in their second attempt, irrespective of the carcass size.

Below we discuss these results in more detail to understand what drives these patterns in age-dependant plasticity, at both a population and individual level and look at the consequences of this age-related response.

Behavioural Plasticity in Parental Care

Previous research has illustrated no effect of carcass availability or quality on provisioning behaviour (direct care), and currently research has only demonstrated that larger carcasses increase pre-natal parental behaviour in burying beetles (Smiseth & Moore, 2002). Yet, this thesis demonstrates that individuals adjusted their level of parental care to carcass size. Additionally, this chapter found that older individuals did adjust parental behaviour and that the response between young and old populations differs only because of the order effect, as the order effect had a substantial effect on how young individuals responded.

There seems an obvious explanation as to why we see a change in how young individuals respond with high residual reproductive value they restrained reproduction in their first breeding attempt and upregulated care in their second attempt. This is supported by the fact they seemed to restrict the number of offspring they produced in their first attempt. Furthermore, the results suggest that females are restraining reproduction rather than responding to the number of offspring (Rauter & Moore, 2004a), as more care was provided on a small carcass in the second attempt, despite more larvae being produced on a larger carcass in their first attempt.

Why old individuals have adjusted parental behaviour to the carcass size however is more perplexing, as it is contrary to the hypothesis that individuals with less reproductive potential are less plastic. Additionally, individuals increased, not decreased, their levels of parental care on a small carcass and this was consistent regardless of the order in which individuals experienced carcasses of different sizes. Previous studies have generally found more care was provided on larger carcasses, due to the higher levels of carcass maintenance involved (Smiseth & Moore, 2002). One explanation for this variation in results is the measure of parental care used in studies of burying beetles. While we can assess how often an individual is observed to provide care we cannot measure the intensity of care within a given interaction. Alternatively, because larger carcasses lead to extended levels of pre-natal investment, by increasing the time spent manipulating and processing the carcass (Smiseth & Moore, 2002), reproductive energy available for post-natal investment, such as provisioning of offspring, may potentially be limited and therefore explain why we saw more care on a small carcass (Smiseth & Moore, 2002). Lastly, what we observe maybe the response to offspring need. Several studies have illustrated how parents adjust parental care in relation to offspring need and begging (Smiseth & Moore, 2002; Rehling *et al.,* 2012). Burying beetles, known to respond to larval begging (Smiseth & Moore, 2002), may therefore also be responding to offspring need, by increasing care in a resource limited environment (small carcass) where they haven't adjusted offspring number in relation to the resource. This increased parental care will consequently improve offspring growth (Eggert, Reinking, & Müller, 1998). This hypothesis however has received mixed support across taxa, with suggestions of a decline (Meunier *et al.,* 2012), constant (Nichols *et al.*, 2012) and an increase (Whittingham & Robertson, 1994) in parental effort in a resource limited environment.

Further analysing this result is important, as understanding why we see plasticity and average levels of parental behaviour alter with age are assumed to be directly related to age, but as demonstrated in this paper it is also dependant on the previous and current breeding environment experienced. Indicating that future research on how age influences parental behaviour needs to consider the affects in multiple different environments.

Life-history Plasticity (Number of Offspring)

The number of offspring females produced in relation to the carcass size was dependant on age: Young females followed previous research, producing more larvae on larger carcasses (Creighton, 2005; Creighton, Heflin, & Belk, 2009; Smith *et al.*, 2015b), but older individuals were less responsive producing similar numbers of larvae irrespective of carcass size, as predicted by theory (Fischer *et al.,* 2014). However, this interaction was dependant on the current and previous breeding environment (order effect). Individuals given a small then large carcass emphasised the prediction, with old females being almost non-responsive and younger individuals increasing their response, compared to individuals that were provided with a large carcass first.

The difference in plasticity with age in relation to the trait number of offspring, I argue is largely based upon how the life-history strategy of individuals alters overtime. Currently, it is predicted that the response to carcass size is an adaptive reproductive strategy to maximise lifetime reproductive success (Smith *et al.,* 2015a). However, classic life-history theory suggests that reproductive investment into current reproduction will increase with age (Williams, 1966; Clutton-Brock, 1984), support for this hypothesis has been found in female burying beetles, in which they terminally invest when they have a low future reproductive potential (Cotter, Ward, & Kilner, 2011; Creighton, 2005). Consequently, an individual's age is likely to alter the costs and benefits of the plastic response to carcass size by influencing an individual's life-history strategy. It would therefore be expected that older individuals would invest into current reproduction as the chance of future reproduction is low. Thus, restraining reproduction on a small carcass in the first breeding attempt will provide no benefit and is potentially costly on lifetime reproductive success, as the chance for breeding multiple times in short lived burying beetles in the wild is rare (Scott, 1998). This would have the consequent effect of reducing the plastic response to carcass size change, as there then would be less energy available for reproduction in the subsequent attempt. However, for younger individuals restraining reproduction on a small carcass is adaptive (Smith *et al.,* 2015b), as the chance of future reproduction is higher and therefore individuals can maximise energetic resources available when potentially breeding on a higher quality resource. This would support why the results in this chapter find that old individuals are less plastic than young individuals.

The complication is that a burying beetle's life-history strategy in not only influenced by age but also the current and previous breeding environment experienced (order effect). Previous work has illustrated that in the first breeding attempt beetles show a greater reproductive restraint on a small carcass than a large carcass (Creighton, 2005). Furthermore, it has been shown that breeding on a low quality resource then a high quality resource induces a terminal investment response in their second breeding attempt but the opposing way means individuals will restrain reproduction in their second attempt (Smith *et al.,* 2015b). Whilst these results explain why we see an order effect on the plastic response in younger individuals, it doesn't explain why we see a change of responsiveness in older females. This is because if older individuals value current reproduction over future investment, then why would individuals restrain reproduction on a small carcass in their second breeding attempt. Instead, we predict this observation is due to a decline in fecundity with age in the second breeding attempt, previously shown in burying beetles (Cotter *et al.,* 2011), however, the extent of the decline is greater on a small carcass, possibly due to the availability of resources for the parental female. This hypothesis however would need further testing.

The Effects on Reproductive Performance

Changes in parental behaviour and number of offspring have implications for reproductive performance in burying beetles. As a result, the fitness (brood mass) of an individual and the quality of offspring produced varies with age, breeding experience and carcass size. Older individuals produced heavier offspring on a large carcass than they did on a small carcass, while young individuals had heavier offspring on a small carcass. Older individuals were always more successful than young individuals in the first breeding attempt, this is because young individuals seemed to restrain reproduction in the first attempt. Furthermore, because the order effect influenced only young individuals, the extent of the difference in reproductive performance between age cohorts was dependant on what carcass size young individuals experienced in their first breeding attempt.

While it is therefore suggested that as burying beetles age there is a decline in fecundity (Cotter *et al.,* 2011), the relative success of older individuals within a population may in turn be greater than young individuals, despite the costs of ageing, as the environment significantly impacts reproductive success by altering an individual's life-history strategy. This result clearly highlights the need to analyse age effects in relation to internal state factors such as individual experience, as well as the external environment and the interaction between them, something often not considered, yet the effects are likely to important and widespread. For example, in mountain goats (*Oreamnos americanus*) social rank, determined by age, influences reproductive success and investment into current reproduction. However, If young individuals gain a higher social rank, the relative investment in current reproduction would differ from the expected hypothesis that reproductive success and investment should increase with age (Clutton-Brock, 1984). Similarly, this chapter found the quality of offspring (mean larval mass) produced in a breeding attempt relied on the age and size of the carcass. This is partly opposing current empirical research, as several papers have suggested older individuals produce lower quality offspring (Wang & vom Saal, 2000; Kern *et al.,* 2001; Descamps *et al.*, 2008). However, this chapter found that while this occurs on small carcasses it doesn't on a large resource, again highlighting the need to study on a multi-dimensional level. This is important as it is argued that producing offspring when older has implications on the fitness on subsequent generations (Wang & vom Saal, 2000) but in our results this doesn't seem the case, and producing offspring when young can also have implications on the quality of offspring.

Individual Plasticity

In the past, there was an assumption that individual plasticity mirrors population level plasticity. However, this is not likely to be the case. Quantifying among-individual variation has therefore become an important element to understand population level plasticity. Additionally, by studying IxE it establishes the variation between individuals in plasticity for which selection acts upon (Nussey *et al.,* 2007). In the current study the results suggest generally older individuals were more variable. Firstly, because Female ID in the older population explained a greater level of unexplained variation, this was especially so for parental behaviour. Secondly, there was more variation among older individuals in their plastic response (IxE), for the number of offspring produced within a brood. This suggests that older individuals do respond but the plastic response is just more variable both in the direction and magnitude, indicating that the conclusion at a population level is misrepresented on an individual level.

One explanation to why females are more variable as they age is based on variation in the decline in fecundity they experience with age. It has often been suggested that a decline in fecundity with age is related to how age affects individuals differently, both over time and on specific traits (e.g. survival or reproductive performance) (Vaupel & Yashin, 1985; van de Pol & Verhulst, 2006; Descamps *et al.,* 2008). Because of this, the investment strategy between individuals will vary, for example how individuals trade-off investment into current reproduction or future reproduction, this would ultimately alter the optimal level of plasticity according to the theoretical model on age-dependant plasticity (Fischer *et al.,* 2014), so while individuals are responding differently, the data in this chapter would still fit the predictions made by the Fischer et al (*2014*) model.

Alternatively, variation in female plasticity is also due to variation in female state/quality. State dependant life-history theory suggests that life-history trade-offs, such as the investment into current vs future reproduction is related to multiple conditions within the individual (McNamara & Houston, 1996). For example, it is demonstrated that a terminal investment strategy is not only due to age but being immune challenged (Bonneaud *et al.*, 2004; Cotter *et al.,* 2011) or, as for burying beetles, related to resource quality (Creighton, 2005). Age is therefore not a direct indicator of the future reproductive potential and reproductive performance of an individual, which are influencing the optimal level of plasticity. Consequently, the direction and degree of plasticity within an individual at any time is likely to vary, especially in older individuals where they have likely to have been exposed to a greater level of environments with which the experience will vary over space and time amongst individuals. Similarly, the increased individual variation in the older population could be because older beetles need to invest more resources into maintaining somatic tissues, thus having less to invest in reproduction, furthermore because individuals deteriorate at different levels the greater individual variation observed in the older population may only be due to somatic noise.

Separating variation in individual condition/state from age effects on life-history strategies impose many challenges in the future. However, it seems fundamentally important if we are to establish how individuals alter investment strategies and how age truly effects plasticity.

Conclusion

The main finding of this chapter is that plasticity in parental behaviour and reproductive investment is age-dependant to a certain extent. However, the results were not as predicted. Instead of finding that old individuals are un-responsive to environmental change (Fischer *et al.*, 2014), this chapter demonstrated that whether they respond to environmental change or not, depends on the trait and the current and past environment experienced. Broadly, this first suggests that age may play a minor role in influencing the pattern of selection on plasticity and evolve, following Fischer's *et al* (2014) predictions. However, because the pattern of selection influenced by the population age will also depend on other environmental variables, in this case the current and past environmental conditions, selection is likely to fluctuate on age-dependant plasticity because of the environmental heterogeneity. Simply meaning evolution of the predicted pattern of age-dependant plasticity will rely on other environmental variables being stable overtime.

Furthermore, this chapter illustrated the importance of studying individuals, as the results demonstrate that older individuals were more variable than younger individuals. As discussed, this chapter suggests that it is likely to be due how age affects individual state/quality and how age affects individuals differently. Additionally, the result indicates that while older beetles were considered less responsive on a population level than younger beetles, the greater variability found amongst individuals indicates that old individuals do respond but that their response is less consistent with other individuals of the same age. This has potential implications to how research should in the future consider individual variation and how selection may act upon it, as the results in this chapter indicate that as individuals age, the expression of plasticity amongst individuals is more environmentally (e.g., individual state) influenced demonstrated by the greater variability found amongst old individuals. This could suggest there is potentially less genetic control over the expression of plasticity overtime. Thus, genetic evolution on plasticity is likely to slow in older individuals, as there is a reduced heritable value to the plastic response, something to further consider and analyse in future research.

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Chapter 4 – General Discussion

The addition of including individual level and multi-dimensional environmental analysis into phenotypic plasticity research has become essential. As discussed in this thesis, we currently know that doing so could potentially demonstrate how natural selection is acting on plasticity and its potential role(s) in evolution (Nussey *et al.,* 2007; Westneat *et al.,* 2009). Empirical focus has consequently grown in the recent past but detailed research often remains rare. However, the rewards are evident when robust conclusions can be made about how selection may be acting on plasticity (e.g., Nussey *et al.,* 2005b). The main aim of this thesis was therefore to simply investigate how female burying beetles respond to environmental change and the implications the response has on reproductive performance. However, this thesis differs from previous studies on burying beetles and other taxa, by considering how individuals responded in a multivariate environment in order to also further broaden our understanding of phenotypic plasticity. Additionally, in both chapters the aim was to answer two independent questions where research on plasticity remains limited.

In chapter 2, the aim was to analyse whether females adaptively adjust reproductive and maternal traits in relation to a change in the competitive environment. It was predicted that if females altered trait expression as a way to increase offspring size in a competitive environment, it would follow predictions made alongside an adaptive response hypothesis (Fox & Mousseau, 1998; Mousseau & Fox, 1998). The results demonstrated that while parental investment declined in a competitive environment, following more closely with a costly competitive hypothesis, female's adaptively adjusted brood size by reducing offspring number in a competitive environment. This meant offspring were consequently larger than when females experienced a non-competitive environment. The chapter concludes that the response in brood size is a form of adaptive transgenerational plasticity which matches offspring phenotypes with the environment (Fox & Mousseau, 1998; Mousseau & Fox, 1998). It would therefore be expected to be selected upon and evolve, assuming the environment consistently fluctuates between a competitive and non-competitive environment, a key factor which favours selection on plasticity over a fixed phenotype.

In chapter 3, the aim was to analyse whether plasticity was age-dependant. Recently, it has been predicted that plasticity becomes decreasingly favoured overtime and as a result older individuals become un-responsive to environmental change (Fischer *et al.,* 2014). This chapter examined this prediction, by looking at how the age of female burying beetles affected their adjustment in brood size in relation to a change in the quality of breeding resource. The results in this chapter demonstrated that age does influence the plastic response and may consequently alter selection pressures acting on plasticity. However, whether on average older individuals respond to environmental change or not depends on the trait and the current and past environment experienced. This meant that only within specific environmental conditions did the results support the predicted pattern that old individuals were un-responsive and young individuals were responsive to environmental change. Furthermore, by analysing individuals the results showed that individuals substantially differed in their plastic response (IxE). This illustrates that old individuals are responsive to environmental change even when the population is regarded as un-responsive. Additionally, the results show that the plastic response amongst older individuals is more variable. As discussed, the chapter argues that it is likely to be due to how age emphasises individual state and quality differences. This could broadly suggest that an individual's response to environmental change will be increasingly determined by how the state or quality of that individual changes overtime rather than its genetic architecture.

Below I discuss these results in reflection to the main aim of this thesis; what has it told us about plasticity and burying beetles that we currently do not know and how has it improved our understanding of how plasticity evolves in burying beetles. Lastly, I discuss how this research has developed our broader understanding of phenotypic plasticity for future research.

4.2 Plasticity, Evolution and Burying Beetles (*Nicrophorus vespilloides*)

Burying beetles are considered highly plastic (see section 1.5, table 1) and our thesis would seem to concur with such a statement. This thesis found evidence of plasticity in both chapters. Furthermore, previous papers researching plasticity in burying beetles have generally concluded that plasticity is adaptive and will evolve to the specific environment and phenotype measured within the study (see section 1.5, table 1).

However, with few papers analysing in a multivariate environment, the research in this thesis shows that the responses are more complicated than previously illustrated in univariate studies. For instance, in chapter 3, at a population level, old individuals under certain environmental conditions were shown to be un-responsive to carcass quality. This is contrary to the previous expectations that all female burying beetles adjust reproductive investment to carcass quality and that the response is positively selected upon (Creighton, 2005; Creighton *et al.,* 2009). Instead we find that because plasticity to carcass quality is further influenced by an individual's age and the previous and current breeding experience, the expected response to carcass quality is not always observed and potentially not always the most adaptive. This could imply that we have been previously overestimating the benefits of a plastic response to an environmental change, and given a biased view of how selection is acting in burying beetles because we have been looking at the response within one environmental dimension. Instead we should consider how multiple environments interact; as chapter 3 found that age influenced the direction and magnitude of the plastic response, which in turn will potentially alter the selective pressures acting on the response, thus altering the evolution of plasticity. By looking at plasticity in multivariate environments we have therefore established that selective pressures acting on plasticity in response to the quality of the breeding resource may fluctuate depending on the current or past environmental conditions and an individual's age. This poses future challenges in research, for example in chapter 2 female's adaptively adjusted brood size to the competitive environment, and whilst we know that carcass size or past experience did not alter the response, what about age? How does being young or old influence the adaptive response to competition and how would this affect selection on this response? Similar questions could be asked about various traits and various environments where plasticity is expected to be under directional selection.

Another main objective of this thesis to further our understanding of plasticity in burying beetles was to analyse at an individual level. By assessing how individuals respond we can quantify variation found amongst individuals and what factors cause individuals to vary. This improves our understanding of how selection may act upon plasticity as it measures the variation amongst individuals for which selection acts upon (see section 1.3, Nussey *et al.,* 2007). In both chapters the results provide evidence that plasticity in reproductive traits vary amongst individuals. Firstly, this highlights that the expected response, illustrated through population level analysis, and the assumption that individuals follow this same response is untrue, clarifying the need to study individual level plasticity. Secondly, the results indicate that there is variation in plasticity for selection to act upon, and assuming the response is genetically heritable, plasticity to carcass quality and competition has the potential to evolve in burying beetles. The next challenge would therefore be to establish whether the responses are heritable using full or half-sib experimental designs, and extending the study into the wild to determine the costs and benefits of the response within a natural fluctuating environment, rather than the fixed conditions of the lab.

Furthermore, this thesis aimed to interpret what causes individuals to vary in plasticity. The thesis found that environmental variables seem to partially determine the level of variation found amongst individuals (IxE) in female beetles. For example, carcass size was manipulated in both chapters, and on a larger carcass the plastic response to environmental change and the phenotypic expression of a trait varied greater amongst individuals than when breeding on a small carcass. This follows expectations that environmental factors as well as genetic architecture determines phenotypic expression and the plastic response within individuals (Nussey *et al.,* 2007), however why on larger carcasses there is more variation amongst individuals and what the implications are remains unknown. One explanation is that variation in individual quality is emphasised on larger carcasses, as only high quality individuals can benefit from the additional resource size, meaning there is greater variation found amongst individuals. This may consequently have potential implications on the strength and magnitude of selection, as fitness differences between individuals would consequently be exaggerated on larger carcasses. In the context of this thesis, it would therefore be expected that the evolution of adaptive transgenerational plasticity would differ between populations breeding consistently on a large or a small carcass, because of how carcass size affects the level of variation in plasticity amongst individuals.

Additionally, this study found individual age also influenced the variation in plasticity amongst individuals (IxE): older individuals varied greater in their plastic response compared to young individuals. Again, this may have implications on how selection is acting on plasticity, and similarly it is likely because the environment, age, is likely to emphasise individual quality. However, to accurately determine how changes in individual variation in plasticity (IxE) impacts selection would require understanding whether larger carcasses or the older population emphasises genetic differences amongst individuals, or individual state/quality differences, which are partially determined by the environment. This is because it will change the heritability of the plastic response, a key component in evolution (Darwin, 1871), otherwise selection may be acting on a population where the trait is environmentally dependant and therefore has little evolutionary potential.

4.2.2 What has this taught us about the evolution of plasticity in burying beetles?

Based upon the results in this thesis and the fact we know that the environment when they breed is highly stochastic due to the breeding resource, I expect evolution to act to make traits in burying beetles more environmentally dependant, reducing constraints on plasticity caused by an individual's genetic architecture. This is because there seems to be no optimal plasticity, as environments constantly interact influencing the direction and magnitude of the response, individuals that therefore can manipulate their plastic response in relation to the current and past environments may consequently be the most successful. This prediction is supported by the fact we see both carcass size and age dictate the level of individual variation in plasticity (IxE), showing traits are at least partially environmentally dependant. It would also explain why phenotypes that are highly associated with individual fitness, e.g., body size, are largely determined by environmental variables (Hopwood *et al.,* 2015). The problem to consider and answer in future research would be can evolution occur on traits to make them less heritable as evolution requires additive genetic variance. Past research however, has shown that environmental heterogeneity degrades heritability (e.g., Simons & Roff, 1994) and that evolutionary processes acting on phenotypes largely depend upon the ecological conditions because the genetic architecture of a population is unstable in temporally fluctuating environments (Robinson *et al.,* 2009). Whilst both these studies relate to the expression of a phenotype rather than plasticity, it seems relevant to extend these conclusions to how the stochasticity of the environment may affect the evolution of plasticity.

4.3 Developing our understanding of Phenotypic Plasticity for the future

Recently, theoretical research on phenotypic plasticity has grown dramatically, one example of this has been determining if the evolution of plasticity is age-dependant (Fischer *et al.,* 2014), something which was examined within this thesis. At first glance the results in chapter 3 agree with Fischer *et al's* (2014) predictions which are supported by similar empirical papers (e.g., Atwell & Wagner, 2014). However, conclusions need to be made carefully. By studying individual variation, chapter 3 shows old individuals are responsive and in fact whether they respond or not depends also on other environmental variables. Further in depth research is therefore required to make broad conclusions about how age truly affects the evolution of plasticity.

However, by studying the effect of age on plasticity it has highlighted a different perspective when interpreting individual responses that is rarely discussed. The age of a population to a certain degree dictates the level of variation in plasticity (IxE) amongst individuals: An old population varies greater than a young population. Furthermore, the quality of the resource (carcass size) was found to have a similar impact. To my knowledge interpreting this finding in other papers is rarely considered. One example however, is a study on red deer, which indicated that at high density populations the phenotypic expression/elevation on a reaction norm varied greater than populations in low density environments (Nussey *et al.,* 2005b). Although the paper doesn't refer to how it alters variation in plasticity (IxE), it does suggest that individual quality causes greater variability in a high density environment, a similar conclusion made within this thesis. It also implies that it has implications on selection, in this case giving an impression that selection is not positively acting on plasticity in high density environments, despite plasticity being beneficial (Nussey *et al.,* 2005b). It is therefore a topic of research that needs future consideration and discussion in relation to future and past studies on plasticity. For example, great tits (*Parus major*) have been studied numerous times in plasticity literature. One paper demonstrating that individuals substantially vary in plasticity (Nussey *et al.,* 2005a), whilst another paper studied in a different location, indicates that there is little IxE, and unlike the above study the population follow the predicted adaptive response closely (Charmantier *et al.,* 2008). The current explanation for this is that it may indicate past selection on the population with little variability, however, according to this thesis it may potentially be due to how an environmental variable that differs between the populations causes greater variability amongst individuals. This is important as the explanation may have little to do with a population's evolutionary history.

Lastly, this thesis has established the importance of looking at how the environment influences population/individual responses to a specific environmental variable. In chapter 3 the results find that both previous and current breeding experience and age alter the plastic response to carcass quality. However, with a vast level of environments to manipulate and the interactions between them to be analysed, gaining a comprehensive understanding of how the environment influences plasticity would require huge amounts of research. Furthermore, it is unknown how closely we can extend the conclusions to populations in the wild. A new approach may therefore be required to analyse the effect of the environment on phenotypic plasticity. One potential method would be to study multiple populations in different locations, and analysing whether both plasticity at a population and individual level differs between them. It would then require establishing what environments differ between the locations and then how that environment(s) alters the plastic response? For example, does it alter the optimal response or the level of variability found amongst individuals. By using this method, you would be determining the environments to manipulate based upon what occurs in wild populations rather than logically selecting environments to manipulate in the lab. However, using this method would first require establishing an initial environment that populations plastically respond to but is stable across the experimental locations, a factor which will only suite a select number of species.

4.4 Conclusion

The main aim of this thesis was to broaden our understanding of plasticity in burying beetles. Chapter two supported findings in a similar study, females reduce brood size and increase larval mass in a female-female competitive environment (Creighton *et al.,* 2009), supporting evidence of adaptive transgenerational plasticity (Fox & Mousseau, 1998; Mousseau & Fox, 1998). In both chapters the results show a significant level of individual variation in plasticity (IxE) in reproductive traits, thus confirming there is variation for selection to act upon and the potential for evolution, one of the first studies to show this in burying beetles. However, this thesis also demonstrated by considering how environmental variables interact (e.g. age x carcass size) influencing the costs and benefits of a plastic response, past conclusions made on the importance of a plastic response which was measured along a single environmental vector (univariate environment), may have been overestimated in burying beetles (i.e. carcass size). This is because in chapter 3 the research established that age and previous experience influence current decisions on how to respond to a change in carcass quality. Furthermore, because burying beetles live in highly stochastic environments a fixed response to carcass quality would limit its ability to adapt, when the adaptive response is likely to fluctuate because of interactions between other environmental variables. Lastly, by studying individuals this thesis has established that carcass size and age influence the level of individual variation in plasticity (IxE) amongst female burying beetles. On larger carcasses and in older populations the degree of variability found amongst individuals was greater. This is something that has rarely been interpreted because of the limitations of population level research. Yet, this may affect how selection acts on plasticity and eventually how plasticity evolves in burying beetles it is therefore something to consider in future research.

To further develop the understanding of plasticity in burying beetles within the context of this thesis, future studies should first consider analysing where the source of variation that I have shown between individuals exists. By doing so we would then be able to establish how much of the variation is made up of genetic and non-genetic components, therefore determining whether the response is heritable, an important element to assess a traits evolutionary potential. While it would have been entirely appropriate to analyse such data within this thesis, our primary aim was to establish the effect of multiple interacting environments on a plastic response to environmental change, and to focus on quantifying variation in plasticity between individuals due to both genetic and non-genetic components, as selection acts on the individual (Nussey *et al.,* 2007). However, completing this research would pave a way into developing our understanding of the mechanisms involved for plasticity to evolve. Recently, research has begun investigating such topics, for example how rapid plastic responses are linked to the genome. For example, one paper has highlighted that individuals can adjust behavioural patterns because of RNA editing through transcriptome plasticity, demonstrating how behavioural responses occur on a genetic level (Liscovitch-Brauer *et al*., 2017). Further to this, recent theoretical work has suggested that behavioural instincts to environments can evolve from selection acting on behavioural plastic responses through an epigenetic mechanism (Robinson & Barron, 2017). This is an interesting topic with many possible applications, as the paper discusses how learnt behaviour maybe passed through generations via transgenerational epigenetics (Robinson & Barron, 2017), and in a similar manner on a long-term basis how culture may form within animal societies. While empirical work remains rare around studies on linking behavioural plasticity to the genome it is certainly a new avenue of research that needs more attention.

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