Effect of nutrient limitation on the Mediterranean flour moth, *Ephestia kuehniella* Zeller

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Santhi Priya Bhavanam

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ABSTRACT

The Mediterranean flour moth, *Ephestia kuehniella* is a cosmopolitan pest of stored products that now has wide distribution in flour/feed mills in New Zealand. Understanding how individual behaviour and life-history strategies evolve in response to environmental variation will help predict the population dynamics and allow us to develop environmentally safe pest control measures.

This study investigated effects of food shortage and the responses this stimulated in *E. kuehniella.* I artificially created food stress environment by rearing *E. kuehniella* larvae at five different population densities of 50, 100, 200, 400 & 800 on a constant amount of diet (50 g). Population density had a detrimental effect on *E. kuehniella* fitness. At higher population density (800) due to food shortage larval period was prolonged, percent survival and pupal mass of both sexes decreased. Poor nutrition during the larval stage also effected adult morphology and reproductive output. Female fecundity decreased with increased population density. Females that developed at high population density (800) emerged with small head, thorax and forewing, but food stressed females developed large abdomens relative to their body mass. There were no significant changes in female ovipositor length in response to nutrient limitation indicating that under poor environmental conditions females allocate more resources to reproduction and in particular to traits that influence offspring. In males, head and thorax width decreased with increased population density. Males at higher population density had large forewings relative to their body mass, possibly to aid movement to new habitats. Genital traits were insensitive to food shortage resulting from crowding. Although males at population densities of 400 & 800 produced fewer eupyrene

sperm they had similar mating frequency and transferred similar numbers of apyrene sperm indicating that male development responds to juvenile environment. Males and females use visual and chemical cues to assess quality of potential mates during mate selection and prefer individuals that developed at high population density (800) compared to low population density (200) when their weights were matched, probably to obtain direct and indirect genetic benefits. *Ephestia kuehniella* obtains indirect genetic benefits through mate choice decisions. Body size has a heritable component and large parents produce large sons and daughters. Mother body mass influences offspring growth rate and daughter developmental period is shortened with increase in mother body mass but no such effect was observed on son developmental period indicating a non-genetic maternal effects. On the other hand, fathers do not have a notable influence on offspring growth rate and as a result the offspring of large fathers took longer to develop. Similarly, sons and daughters of polyandrous and cross culture females were heavier and polyandry increased female fitness especially in stressful conditions.

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This thesis consists of 8 chapters – General Introduction (Chapter 1), six experimental chapters (Chapters 2–7) and a General Discussion (Chapter 8). Chapters 3–7 are formatted ready for submission to peer-reviewed science journals and Chapter 2 has already been published. For this reason, each chapter is a standalone with its own Abstract, Introduction, Materials and Methods, Results and Discussion and hence there is some repetition in the chapters. For every chapter the table and figure numbering refreshes but each chapter has distinct running header for clarity. A single combined reference section is presented after Chapter 8.

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CHAPTER 1

General Introduction

Post-harvest losses due to stored product insect pests were estimated to be 9% and > 20% in developed and developing countries, respectively (Pimentel, 1991). The losses may be primarily due to product infestation and secondarily due to contamination and secondary pest attack (Phillips and Throne, 2009). Most of the stored product pests belong to order Coleoptera and Lepidoptera. In Lepidoptera alone, 70 moth species from several families Pyralidae, Tineidae, Oecophoridae and Gelechidae (Cox and Bell, 1991) are identified as stored product pests. Some of the most serious economic pests among them are the Mediterranean flour moth, *Ephestia kuehniella,* the Indian mealmoth, *Plodia interpunctella*, the Angoumois grain moth, *Sitotroga cerealella*, the almond moth, *Cadra cautella* (Cox and Bell, 1991).

Ephestia kuehniella, Mediterranean flour moth is a cosmopolitan stored product pest. The survey result of 1986 indicates the wide distribution of Mediterranean flour moth in flour/feed mills in New Zealand. Larvae particularly feeds on flour but also infest a wide range of commodities (Hill, 2002). Larvae spin silken threads while moving, to which food particles are attached, forming silken webs that clog machinery (Subramanyam and Hagstrum, 1996). The ability of the Mediterranean flour moth to thrive well on low moisture content food and develop faster in heated premises such as flour mills (Jacob and Cox, 1977) contributes to its success as a stored product pest. Egg hatching can occur from 12 to 31°C, irrespective of relative humidity. Last instar larvae may enter diapause triggered by temperature, photoperiod or nutrition (Subramanyam and Hagstrum, 1996). Though control is achieved with insecticides, their frequent usage may result in accumulation of insecticide residues in food and environmental pollution posing human health hazards. Alternative environmentally safe control practice includes reduction of moisture content in food, use of food repellents and controlled and modified temperature (Phillips and Throne, 2009). In crop pests and natural enemies, individuals exhibit phenotypic plasticity to fluctuating environmental conditions. We do not know how stored product pests are affected by environment variation used as one of the control measure. Understanding the behavioural response to environmental variation helps understand its life-history strategy and sexual selection mechanisms. A good knowledge on these factors may also help refinement of pest management tactics.

Importance of this study

In many insects food is consumed in different ways during different life stages and individuals may experience nutrition limitation during some or all life-stages. Depending on the life-stage and dietary difference between stages, the effect and severity of food shortage on life-history traits differs (Boggs, 2009 and references therein). If food shortage persists for a long period of time then individuals alter their developmental, physiological and behavioural processes in order to survive (Metcalfe and Monaghan, 2001). This process has been categorized into three phases (Monaghan, 2008): (1) Tolerance phase, organisms reduce fitness loss through phenotypic plasticity. Examples include prolonged developmental time and compensatory growth (Roff, 2002). (2) Mitigation phase, trade-offs, selective allocation of resources to one trait rather than others to minimize fitness loss (Metcalfe and Monaghan, 2001). (3) Pathogenic phase, when fitness can no longer be maintained. The effects of these may extend to future generations and lead to changes in population dynamics (Mousseau and Fox, 1998).

Any environmental change that reduces individual fitness is defined as stress (Koehn and Bayne, 1989). However, Hoffmann and Parsons (1991) suggested that even under favourable conditions, maximum fitness is not attained by an individual and hence the stress term should be used only when the survival and reproduction are drastically affected by environment, hence this can only be measured *a posteriori* (Bijlsma and Loeschcke, 2005). Stress responses help individuals survive in fluctuating environments. Differences in ecological requirements and life-history strategy allow individuals to respond in various ways, but many aspects of the response to stress such as food shortage, predation, competition, temperature are not well-studied (see Boggs, 2009). Understanding stress responses is especially important given the likelihood that many species will be affected by global environmental change (Boyce et al., 2006). Even without anthropogenic influences organismal response to changing conditions is fundamental to evolutionary ecology (Bijlsma and Loeschcke, 2005; Boyce et al., 2006 and references therein).

Life-history theory

Life-history theory deals with the organisms' anatomy and behaviour and associated processes such as growth, development, reproduction and death, which are shaped by natural selection. The concept of trade-offs is central to present thinking about the evolution of life-history traits (Roff, 2002), especially where survival and fecundity intersect. As increased fecundity requires large body size, individuals have to prolong their developmental period or increase growth rates to maximize fecundity but both these traits reduce individual longevity. Alternatively, large egg number might be achieved at the expense of egg size but offspring developed from small eggs have lower survival (e.g. Jann and Ward, 1999; Murphy et al., 1983; Richards and Myers, 1980). Therefore, the trade-off with developmental period or egg size limits the fecundity. As different species have different life-histories, trade-off between different traits may vary in a manner that suits the species life-history. The particular trade-off that increases the individual fitness is favoured by natural selection and maintained in the population until mutation and/or gene recombination occurs or selection pressures change stable combinations, called evolutionary stable strategies (ESS's) (for review see Roff, 1992; Stearns, 1992).

Nutrition

Nutrition is essential for an organism to survive, grow, develop (e.g. Bauerfeind and Fischer, 2005) and reproduce (e.g. Braby and Jones, 1995). The quality and quantity of eggs is constrained by nutrient availability. Diet quality and quantity also affects the male gift giving ability (e.g. Wedell, 1994) and accessory gland products (reviewed in Ivy, 2007). Hence, nutrition can shape significantly the strength and direction of selection on mating responses and reproductive strategy (Fricke et al., 2010).

Organisms acquire nutrients through foraging activity. Species-specific foraging behaviour occurs due to past selection that is then genetically, hormonally and neurally regulated (Page and Erber, 2002 references therein). Foraging activity usually does not differ between life-stages in hemimetabolous insects as both nymphs and adults feed on the similar food to a degree dependent of their size and development, but in holometabolous insects, larval and adult feeding substances and rates differ. In Lepidoptera, for example, larvae feed on particular materials depending on the species and obtain all vital nutrients such as protein, carbohydrates and lipids that are used in subsequent life-stages (see Boggs, 2009). Adults of some species feed on nectar (e.g. *Pthorimaea operculella*), pollen (e.g. *Heliconius hecale*) or sugars (e.g. *C. cautella*) while few others do not feed at all (e.g. most species of Bombycidae, Saturniidae, Hepialodae and short-tongued Sphingids) (Jervis et al., 2005 and references therein). In some species, sex-specific feeding occurs as females obtain nutrients from males in the form of nuptial gifts (e.g. spermatophore in Lepidoptera (reviewed in Vahed, 1998)) and this may have substantial impact on foraging activity of different sexes. In addition, foraging activity can also help to reduce predation, pathogen attack and mate location (Savalli, 2001).

Resource allocation

The partitioning of resources among life-history traits influencing their expression, which in turn can alter population demography (Boggs, 1992). To suit particular life-history and foraging demands, resource allocation patterns vary between species depending on the timing of resource acquisition, stage-specific dietary requirement and nutrient availability and within a species, with age and sex (reviewed in Boggs, 1992). For example, among male butterflies that provide nuptial gifts, young males that mate more frequently have greater nitrogenous demands compared to older males and the latter has greater nitrogen requirement than females. This contributes to variance in resource allocation (Boggs, 1997; Boggs and Jackson, 1991; Vahed, 1998).

A simplified life-history model represents the way acquired resources are allocated between the two life-history traits, survival and reproduction (deJong and Vannoordwijk, 1992). In this, the two arms of Y represent survival and reproduction and stem is the amount of resources acquired. An increase in allocation in one arm results in decrease in allocation to the other arm leading to competition for resources. Hence, depending on the species, investment in survival and reproduction varies. If survival is important then resources are divided among survival, maintenance and reproduction (Boggs, 1992). If reproduction is the priority then an all-in-one strategy is followed where the majority of resources are invested in reproduction.

The complexity of resource allocation has led to the development of a model framework for holometabolous insects (Boggs, 2009), in which three stages of resource allocation are recognised: (1) egg and larval nutrients are allocated for larval growth, development and survival; (2) larval nutrients are used for larval foraging, adult soma, non-soma and stored reserves; and (3) adult resources are used for adult maintenance and reproduction (Figure 1).

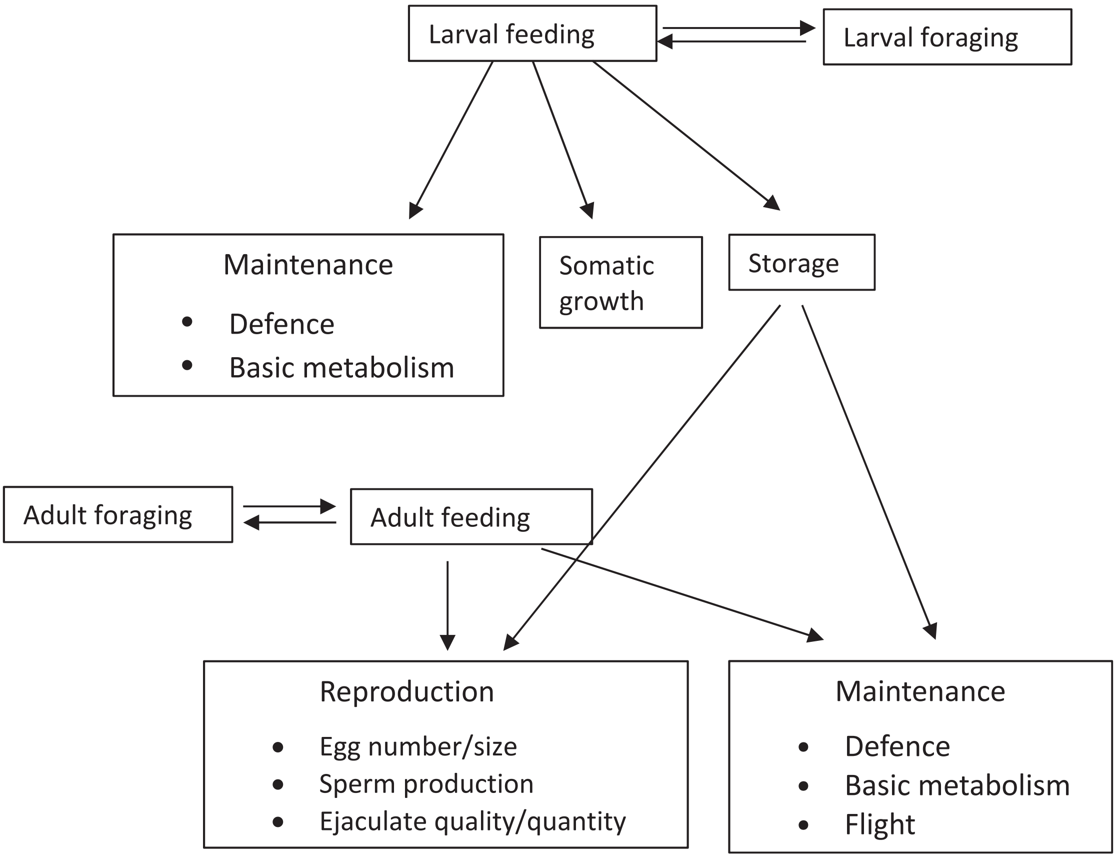

Figure 1. A diagrammatic representation of nutrients obtained during larval and adult stage allocated to various life-history traits in holometabolous insects (based on Boggs, 2009).

Interaction of reproduction with other life-history traits

a. Lifespan

In insect orders, Lepidoptera, Trichoptera, Hymenoptera, Diptera, adult females have mature eggs when they emerge from pupa (Honek, 1993), and the proportion of mature eggs an individual carries at emergence (initial egg load) to lifetime potential fecundity is defined as the Ovigeny Index (OI) (Jervis et al., 2001). Hence, OI varies between 1 and 0 for short and long-lived species, respectively. Species with high OI invest more resources in early reproduction compared to those with low OI. This limits the availability of resources for survival.

b. Mobility

High OI tends to limit female dispersal. Firstly, because of greater wing loading due to greater abdominal mass (Chew and Robbins, 1984) and secondly, development of large or more reproductive organs reduces the space available for development of flight muscles (Tweedie, 1976) and more space allocation to the latter may incur costs on reproduction to females. For example, in female *Heliconius* butterflies, there is a positive correlation between body size and lifetime fecundity. A decrease in ovarian tissue due to decrease in available space results in reduction of female fecundity (Dunlappianka, 1979).

c. Oviposition behaviour

Optimal host range and clutch size is a function of egg load (Mangel, 1989; Parker and Courtney, 1984). In Lepidoptera OI exerts its influence on oviposition substrate selection (Jones, 1977; Root and Kareiva, 1984) and clutch size (Godfray, 1987) through its effects on dispersal (Jervis et al., 2005). Although direct evidence is limited, in Lepidoptera, the polypahgous Geometrids and Lymantrids are found to have OI = ~ 1, whilst monophagous butterflies and sphingids have OI = 0 (Jervis et al., 2005). The chances of finding suitable hosts may affect the clutch size (laying eggs singly or in clusters). For example, moths which lay eggs in clusters have OI approximately close to 1, whereas singly laying eggs as butterflies OI is equal to 0 (Jervis et al., 2005). Likewise, in *Drosophila melanogaster* females that have fewer mature eggs preferred to deposit them on high quality host diet while those with high egg load (>50 mature eggs) accepted low quality host (Courtney et al., 1990).

Evolution of offspring number

At the time of oogenesis, available resources have to be allocated among egg number, egg size and oviposition (Jervis et al., 2008). Reproductive plasticity with regard to these traits is advantageous as it helps to increase the current and future reproductive returns (Roff, 2002; Stearns, 1992). Offspring number is a function of egg quality and oviposition rates and three pivotal factors can be identified (Messina and Fox, 2001).

Lack (1947) postulated that, in altricial birds the number of young fledged per nest was a function of the clutch size and the number of offspring that survive in each clutch. If nestling survival decreases with increasing clutch size then a clutch size that maximizes both offspring number and survival is preferred.

Optimal foraging theory applies to offspring number. Females of some species disperse eggs over a range of hosts and Charnov and Skinner (1984) argued that a female should only deposit the number of eggs that the resource patch can support. Any increase beyond this causes female fitness to fall drastically. A female should leave the resource patch when fitness benefits are likely to decrease but can increase fitness by moving to other resource patches. As this requires searching for additional resource patches, female egg load and internal physiological state are implicated.

There is conflict between the interests of females and their offspring in regards the optimal number per reproductive cycle (Mock and Parker, 1998). Individual offspring fitness is small in large clutches due to sibling competition. In contrast the female interests are maximised when resources are equally distributed among her offspring. If sibling competition is high then parent optimum will be lower compared to clutches without competition. Whatever the circumstance, the match between strategy and fitness outcome depends on accuracy of resource assessment.

Oviposition behaviour and offspring fitness

When, where and how eggs are deposited determines the pre and post-hatching offspring environment. These effects are called maternal effects (Mousseau and Fox, 1998). When multiple eggs are laid in one place, the less mobile young have to compete with each other for resources, and this is most severe if the available resource quality is poor (Fox, 1994). Crowded conditions increase juvenile development time, reduce individual body mass, survival and adult reproductive output and lifespan (e.g. Fantinou et al., 2008; Macia, 2009). Moreover, decreased food availability also affects dispersal ability in flying insects (Hughes et al., 2004). Weak offspring are more prone to pathogen and predator attack and have reduced immune response (e.g. McVean et al., 2002). All these factors contribute to decreased offspring survival that in turn reduces the female reproductive fitness.

Adult morphology

At metamorphosis, resources are allocated to the imaginal discs specific to each adult body part to form the adult phenotype (Chapman, 1998). In insects, larvae obtain nutrients then pupate and metamorphose into adults (Shingleton et al., 2007). When the required amount of nutrients for development is obtained, larvae stop feeding and no further growth occurs. This is the critical size/weight and determines adult size. Once the critical weight is obtained, the release of Juvenile Hormone (JH) ceases and Prothoracicotropic hormone (PTTH) is secreted and larval feeding terminates. The time lapse between achievement of critical size and feeding termination is referred to as the Interval to Cessation of Growth (ICG). During ICG, organ development occurs once body size has been established. Imaginal cells that are specific to each adult body part formed at embryogenesis proliferate to form imaginal discs. During the pupal stage, the imaginal discs protrude to form the respective adult body parts that then attach to the pupal cuticle. Division of imaginal discs starts and terminates at different times and rates that vary intra-specifically and inter-specifically between the discs (D'Amico et al., 2001). Thus, resource allocation at larval and pupal stage also determines the adult body structure and the allometry of adult body parts.

Three different types of allometry are recognised (Cheverud, 1982): (1) ontogenic allometry: it is a growth trajectory that explains the relationship between organ size and body mass during an individual growth; (2) Evolutionary allometry: it explains the relationship between organs among individuals of same age but across species; and (3) static allometry explains the relationship between two organs or between a single organ and body mass among individuals of same species. Allometry between body parts differs as the time and rate of body part development differs (D'Amico et al., 2001) and are sometimes influenced by external environment. The variation in allometric relationship affects the efficiency of the particular organ and has constraints on life-history and foraging traits (Schmid-Nielsen, 1975; Schmidt-Nielsen, 1984). In holometabolous insects, resource allocation to adult body parts occurs after termination of larval feeding and during the pre-pupal and pupal stage. The growth model shows that in a closed pupa changes in resource allocation results in correlated changes in trait development in social insects (Nijhout and Wheeler, 1996). This could occur between traits that are separated far apart (Moczek and Nijhout, 2004).

Sexual selection

Sexual selection is defined as the selection of characters that give certain individuals a mating advantage over the others of the same sex (Darwin, 1871). The outcome is increased number of mating and so reproductive success in each male. However, traits favoured by sexual selection can have a negative effect on survival and time available for an individual to reproduce that can in turn result in the production of fewer offspring by an individual, concept of natural selection. Hence, sexual selection is included under natural selection as a subset.

Sexual selection sometimes results in sexual dimorphism, a phenomenon where males and females of the same species have different phenotypes. The fundamental difference occurs in their reproductive organs and the difference in male and female gamete morphology and reproductive investment. Female gametes (eggs) are large and expensive compared to cheap male gametes (sperm) (Trivers, 1972). If all else is constant, reproductive success of females is limited by egg production while that of males is dependent on mating frequency, which increases proportionally with every additional mating he achieves (Bateman, 1948). In a single mating, each male transfers a large number of sperm that is sufficient to fertilize the entire female egg load. On the other hand, females mate once or twice in their lifetime and are either unreceptive or unavailable during oviposition resulting in a male-biased sex ratio. Males whose reproductive success increases with mating frequency have to compete against each other for females, providing an opportunity for the latter to choose. As a result reproductive success between males differs leading to increased selection pressure on males to develop traits that enhance their mating success (Savalli, 2001).

Mate choice evolves in response to selection for a desired trait or indirect selection due to genetic correlation with a desired trait (Andersson and Simmons, 2006) leading to non-random matings and fertilizations (Edward and Chapman, 2011). The ecological costs of mate choice involve time and energy costs and exposure to predation so the benefits should outweigh the costs. Female choice evolves through natural selection for increased fecundity and offspring survival and the fitness benefits of mating with certain males that can provide territory & oviposition sites, nuptial gifts, more sperm, paternal care (for review see Arnqvist and Nilsson, 2000; Fedorka and Mousseau, 2002) or indirect genetic benefits that increase offspring fitness through good genes, compatible genes and/or genetically diversified genes (Jennions and Petrie, 2000; Zeh and Zeh, 1996).

Males exert choice when there is difference in female reproductive quality, increased mating effort and investment and mate availability and male mating capacity (Bonduriansky, 2001). This can occur before mating where males preferentially court some females more than others, and during copulation where males assess female quality after physical contact and strategically allocate sperm (for more details see Edward and Chapman, 2011). Although this increased investment increases a male’s attractiveness towards females or aggressiveness against rival males it reduces his future reproductive capacity and survival (e.g. Hunt et al., 2004).

Thesis outline

The overall aims of this research were to determine the effects of food shortage on life-history traits, morphology, behaviour and reproductive success using experimental manipulation of the Mediterranean flour moth, *Ephestia kuehniella.* I examined the changes in individual morphology or life-history strategy that occur as a response to changes in environment and the indirect genetic benefits derived from female mating preferences in absence of direct benefits using *E. kuehniella* as a model insect.

Food shortage during the juvenile stage can have detrimental effects on subsequent life-stages and lowers individual fitness. Resource allocation, a function of environmental stress, degree of phenotypic plasticity and genetic variance in resource acquisition strategy is altered to cope with harsh environmental conditions in many insect species (e.g. Boggs and Freeman, 2005; Chippindale et al., 1998; Messina and Fry, 2003). It is unknown, however, if *E. kuehniella* exhibit similar phenotypic plasticity to food shortage and if this strategy suits its life-history and prevailing environment. Due to differences in male and female reproductive physiology and strategies sex-specific buffering effects to food shortage occurs in insects (e.g. Boggs and Freeman, 2005; Jannot et al., 2007). Reproductive success is achieved when an organism responds optimally to prevailing environmental conditions and partitions available nutrients into different traits that add up to form a reproductive strategy. Increased resource allocation to one trait decreases the resources available to other traits (Nylin and Gotthard, 1998) and some traits exhibit plasticity while others do not. There is some evidence of reproductive plasticity in male and female *E. kuehniella* to food stress. Does this have an equal impact on reproductive success of both sexes? Poor nutrition reduces individual condition and in turn expression of condition-dependent sexually selected traits that provide information about mate quality is affected. Even in normal environment marked differences between individuals in terms of size exists and one probable reason is variation in resource acquisition ability of animal. We still know very little about how the mate choice decisions were influenced by multiple environments when controlled for individual condition. The potential benefits to males and females from mate choice decisions in such situations are open to debate. Some studies have found evidence of a heritable component of traits that increase reproductive success. Body size is an important trait that positively correlates with several life-history traits of an individual. However, whether offspring size phenotype is genetically-controlled and/or influenced by non-genetic maternal and paternal effects is still unknown in species where maternal and paternal care is absent.

In the few studies conducted on insects that confirm female preference for novel rather than previous male when remating it has been hypothesized that this is because it results in increased genetic diversity in the clutches (Liu et al., 2014), but there are few data to test this. Most of research in this area has been on frogs, birds and marine animals and has reported marked differences in offspring life-history traits within a single (Aguirre and Marshall, 2012) or later generations (Power and Holman, 2014) but not all (Byrne and Roberts, 2000). Experiments in normal and stressful conditions over several generations found that polyandrous clutches persist for longer periods in beetles (Agashe, 2009). Equivalent studies in species where males do not provide direct benefits are clearly absent in insects.

Model insect

Ephestia kuehniella, Mediterranean flour moth belongs to the order Lepidoptera and the family Pyralidae (Zeller 1879). It is an important pest of stored products (Cox and Bell, 1991; Hill, 2002). As is typical of Lepidoptera, there are four stages in the lifecycle of *E. kuehniella*: egg, larva, pupa and adult. Females deposit eggs inside the food material. Eggs are small, white and oval in shape and just before hatching develop a light yellowish or brownish colour and (Kamel, 1967). Larvae feed particularly on flour but also infest a wide range of food stuffs (Hill, 2002). Initially pupae are light green in colour and turn brown towards later stage of the pupae. Mature pupae are darken followed by adult emergence within 24 h (Subramanyam and Hagstrum, 1996).

Adults are relatively large moths with a wing span of 2.0–2.5 cm. At emergence, females carry few mature eggs and low pheromone quantities (Norris, 1934) and mating occurs within 24 h of emergence (Calvert and Corbert, 1973). During calling, a prerequisite for mating, females lift the abdomen with a protruding ovipositor, releasing the sex pheromone (Dickins, 1936). Males approach the calling female by fanning wings (Traynier, 1968).

Mediterranean flour moth is widely used for mass-rearing of biocontrol agents that predate them (e.g. *Trichogramma evanescens*, Brower, 1984; *Trichogramma aurosum*, Samara et al., 2008) and also used for research into insect behaviour and biochemistry (Corbet, 1973; Rahman et al., 2007). The short life-span (Gonzalez-Nicolas, 1966), simple diet (Cox and Bell, 1991), early sexual maturity (Calvert and Corbert, 1973), large female fecundity and non-feeding adult stage (Norris, 1934) make the species a convenient model.

Experimental conditions of food shortage early in life can be achieved by rearing juveniles at different population densities on constant amounts of food. I reared moths in *ad libitum* and in stressful dietary conditions and measured various parameters related to life-history, morphological and behavioural activities to estimate the effect of nutritional limitation.

The main questions I addressed were:

1. Does nutritional limitation affect life-history traits (chapter 2)?
2. Do females that experience food shortage at juvenile stage alter their resource allocation pattern and produce a phenotype that suits the local environment (chapter 3)?
3. Do males respond to nutritional limitation and are there changes in sperm production, allocation, mating frequency and adult phenotype (chapter 4)?
4. Do mate choice decisions vary with the prospective mates' environment when controlled for condition (chapter 5)?
5. Do offspring inherit their parents' body mass and do parents affect the offspring early development (chapter 6)?
6. Does mating with different males increase offspring fitness in normal and/or stressful environments (chapter 7)?

CHAPTER 2

Effect of nutritional stress through larval crowding on survival, development and reproductive output of the Mediterranean flour moth, *Ephestia kuehniella* Zeller

A version of this chapter has been published as:

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Abstract

Eggs of the Mediterranean flour moth, *Ephestia kuehniella* are widely used for rearing insect biocontrol agents. Understanding how nutritional stress and larval crowding affect its fitness is essential to the development of an optimal production programme. Such knowledge could also help lead to novel management strategies because it is an important storage pest. The development, survival and reproductive output of *E. kuehniella* under five densities (50, 100, 200, 400 and 800 larvae reared on 50 g of diet) were examined. As population density increased, the larval developmental period significantly increased from 27.8 to 33.7 days and the larval percent survival decreased from 77.6 to 47.6%. In addition, the pupal mass of males and females decreased from on average 24 to 16 mg and 28 to 18 mg, respectively. Fecundity of the resulting females reduced significantly from on average 326.8 to 132.6 eggs, respectively. There was no significant difference in fertility, percent pupation, emergence and survival between densities of 50, 100 and 200 larvae. Therefore, the optimal rearing density could be up to 200 larvae on 50 g of diet, but above that food stress becomes apparent.

Introduction

The Mediterranean flour moth (*Ephestia kuehniella* Zeller) is an economically important pest of a wide range of stored commodities throughout the world. Despite its destructive nature, this species is widely used as food for the mass rearing of *Trichogramma* species used as biological control agents (e.g. Brower, 1984; Samara et al., 2008). This requires a well-established laboratory colony.

Previous studies have revealed that nutritional stress and crowding during juvenile stages effect various fitness components in many insect species. For example, larvae of the midge *Chironomus riparius* reared at higher densities have decreased survival rate (Hooper et al., 2003), a prolonged larval period in pink stalk borer *Sesamia nonagrioides* (Fantinou et al., 2008), reduced growth rates and body size of the tropical butterfly *Bicyclus anynana* (Bauerfeind and Fischer, 2005) and lower fecundity in boll worm *Earias vittella* (Tripathi and Singh, 1990). Larval crowding in *E. kuehniella* is also suspected to lead to cannibalism of early instars (Savoldelli, 2006). Xu et al. (2007) reported that adequate adults of satisfactory quality could be obtained with minimal labour from colonies where 100 larvae were reared on 50 g of diet. However, the wide gap between alternative rearing densities in that study (1, 100, 500 and 1000 larvae) limited precision in identifying optimal rearing density difficult (Xu et al., 2007). In the present study, the effect of five larval densities on four fitness parameters was examined in *E. kuehniella*, in order to determine the best rearing practice for this species, and to provide fundamental information for further experimental manipulations of this species reproductive behaviour.

Materials and methods

Insects

A founding population of *E. kuehniella* was collected from feedstock at a poultry farm near Foxton, New Zealand. Colonies were established in plastic containers (8 cm in diameter × 10 cm in high) by rearing 100 larvae on 50 g of diet comprising wholemeal wheat flour (43.5%), maize meal (43.5%), brewer's yeast (3%) and glycerine (10%) (Lima-Filho et al., 2001; Xu et al., 2007) at 25 ± 1°C and 60 ± 10% relative humidity with 14:10 h (light:dark) cycle. All experiments described were carried out in the same plastic containers under these environmental conditions. Larvae were provided with crumpled paper towel (25 cm × 25 cm) for pupation and containers were covered with double-layered nylon mesh for ventilation and exclusion of parasitoids.

Larval densities

To establish five larval rearing densities, eggs were collected within 24 h of laying from a number of females held in plastic containers lined with a porous plastic sheet as an oviposition substrate. Preliminary incubation tests showed that the hatching success of this species was high (96 ± 0.3%). Therefore, to obtain densities of 50, 100, 200, 400 and 800 larvae, 52, 104, 208, 416 and 832 eggs randomly selected from the breeding colony were assigned to different treatment groups. For each replicate, the eggs were placed in a plastic container containing 50 g of diet. Unlike the larvae of many insect species including other moths that live in the open air, *E. kuehniella* larvae live and feed inside their food (store products such as flour). Therefore, the amount of food more or less reflects available living space. In this experiment, 50 g of food occupied about 1/4 of the container space and the top of the container was covered with mesh for ventilation. As a result, the containers did not limit space available to larvae or cause them stress. To minimise variation in egg period, eggs used for each replicate were collected on the same day. Ten replicates of each of the 50, 100, 200 and 400 population densities and eight replicates at 800 population density were established.

Survival, development, pupal mass and fecundity

Pupae were collected daily from all replicates of each treatment and placed individually in glass vials (2 cm in diameter × 7.5 cm in high). The larval developmental period (hatching to pupation), percent pupation (number of pupae/number of neonate larvae), percent emergence (number of emerged adults/number of pupae) and percent survival (number of emerged adults/number of neonate larvae) were recorded. Thirty pupae were randomly selected from each treatment and weighed individually using an electronic dual range balance (Mettler AE100, Mettler-Toledo Inc., Switzerland) with a precision of 0.1 mg.

To compare the effect of larval rearing density on female fecundity (total number of eggs laid) and fertility (total number of fertile eggs laid), 20 newly emerged virgin males and females from each treatment were randomly collected and paired, placing each pair in a plastic container for their lifetime. Eggs were collected daily from each pair until the female died. Harvested eggs were placed in 8.5 cm diameter Petri dishes for incubation at 25 ± 1°C and 60 ± 10% relative humidity. After three days of incubation, eggs were observed for fertility under a dissecting microscope (Olympus SZ III, Japan), with those eggs having black dots (larval heads) being counted as fertile.

Statistics

All statistical analyses were conducted using SAS version 9.3. A goodness-of-fit test was carried out to test the distribution of data. Data on percent pupation were not normally distributed even after transformation and thus were analysed using the non-parametric Kruskal-Wallis test followed by Dunns' procedure for multiple comparisons. Other data were normally distributed and analysed using ANOVA followed by Tukey's HSD test for multiple comparisons. Percentage data were arcsine square root transformed before analysis. The hypothesis rejection level was set at $P < 0.05$.

Results

The larval developmental period at a population density of 50 larvae was significantly shorter than that at a density of 800 larvae, however the larval developmental periods at densities of between 100 and 400 larvae were between the two extremes and not significantly different among themselves ($F_{4,43} = 70.74$, $P < 0.0001$) (Table 1). Percent pupation was significantly higher at densities up to 200 larvae than at densities of 400 and 800 larvae ($F_{4,43} = 15.88$, $P < 0.0001$). Percent survival was not significantly different between densities of 50 to 400 larvae, but these were significantly higher than the percent survival at density of 800 larvae ($F_{4,43} = 12.50$, $P < 0.0001$) (Table 1). For all rearing densities percent emergence did not differ significantly ($F_{4,43} = 0.51$, $P > 0.05$) (Table 1).

Table 1. Mean (±SE) larval (developmental) period (days), and percent pupation, emergence and survival of *Ephestia kuehniella* reared at five densities. Means in each columns followed by the same letter are not significantly different at $P = 0.05$.

Density	Larval period	Percent pupation	Percent emergence	Percent survival
50	27.80 ± 0.16 a	89.60 ± 1.65 a	86.67 ± 2.10 a	77.60 ± 2.15 a
100	28.36 ± 0.15 ab	86.90 ± 5.37 a	84.00 ± 4.02 a	72.30 ± 5.06 a
200	28.97 ± 0.33 b	86.40 ± 2.68 ab	83.04 ± 1.46 a	71.71 ± 2.50 a
400	28.89 ± 0.13 b	80.28 ± 2.19 b	86.58 ± 1.80 a	69.47 ± 2.27 a
800	33.69 ± 0.48 c	56.89 ± 1.83 c	83.60 ± 1.36 a	47.59 ± 1.66 b

For both sexes, pupae resulting from lower population densities of 50, 100 and 200 were significantly heavier than those from population densities of 400 and 800 (Male: $F_{4,145} = 26.38$, $P < 0.0001$; Female: $F_{4,130} = 33.99$, $P < 0.0001$) (Table 2). Females were significantly heavier than males in all population densities tested (50: $F_{1,58} = 32.66$, $P < 0.0001$; 100: $F_{1,43} = 7.83$, $P = 0.008$; 200: $F_{1,58} = 14.53$, $P = 0.0003$; 400: $F_{1,58} = 7.06$, $P = 0.010$; 800: $F_{1,57} = 4.35$, $P = 0.041$) (Table 2).

Table 2. Mean pupal mass (mg) of male and female *Ephestia kuehniella* reared at 50, 100, 200, 400 and 800 population densities. In each column different letters indicate significant difference among the treatment groups ($P < 0.05$). Within each treatment group, females were significantly heavier than males.

Population density	Male	Female
50	23.55 ± 0.55 a	27.84 ± 0.51 a
100	23.02 ± 0.48 a	25.29 ± 0.62 ab
200	21.59 ± 0.61 ab	24.69 ± 0.54 b
400	19.65 ± 0.44 b	21.69 ± 0.63 c
800	16.79 ± 0.58 c	18.90 ± 0.70 d

The fecundity and fertility of adult females decreased with increased population density (Fecundity: $F_{4,95} = 29.91$, $P < 0.0001$; Fertility: $F_{4,95} = 20.63$, $P < 0.0001$) (Figure 1). Although fecundity of female *E. kuehniella* from the lowest population density (50) was the highest, there was no significant difference in fertility (the realised reproductive output) of adult females reared at densities of 50 to 200 larvae ($P > 0.05$). Although there is a strong correlation between fecundity and fertility at all densities this correlation was slightly lower at density 50 compared to the rest (Figure 2). When the population density reached 400, both fecundity and fertility significantly decreased ($P < 0.0001$) (Figure 1).

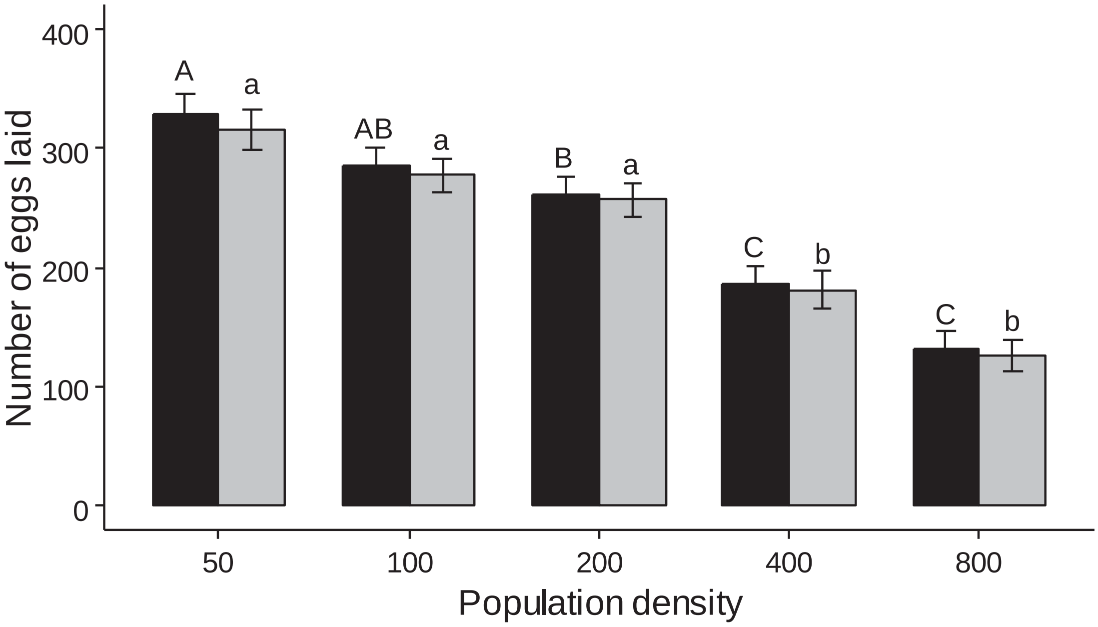

Figure 1. Mean total number of eggs, black bars and fertile eggs, grey bars laid by *Ephestia kuehniella* females developed from five different population densities (50, 100, 200, 400 & 800) under controlled conditions. Bars with the same letters are not significantly different at $P = 0.05$.

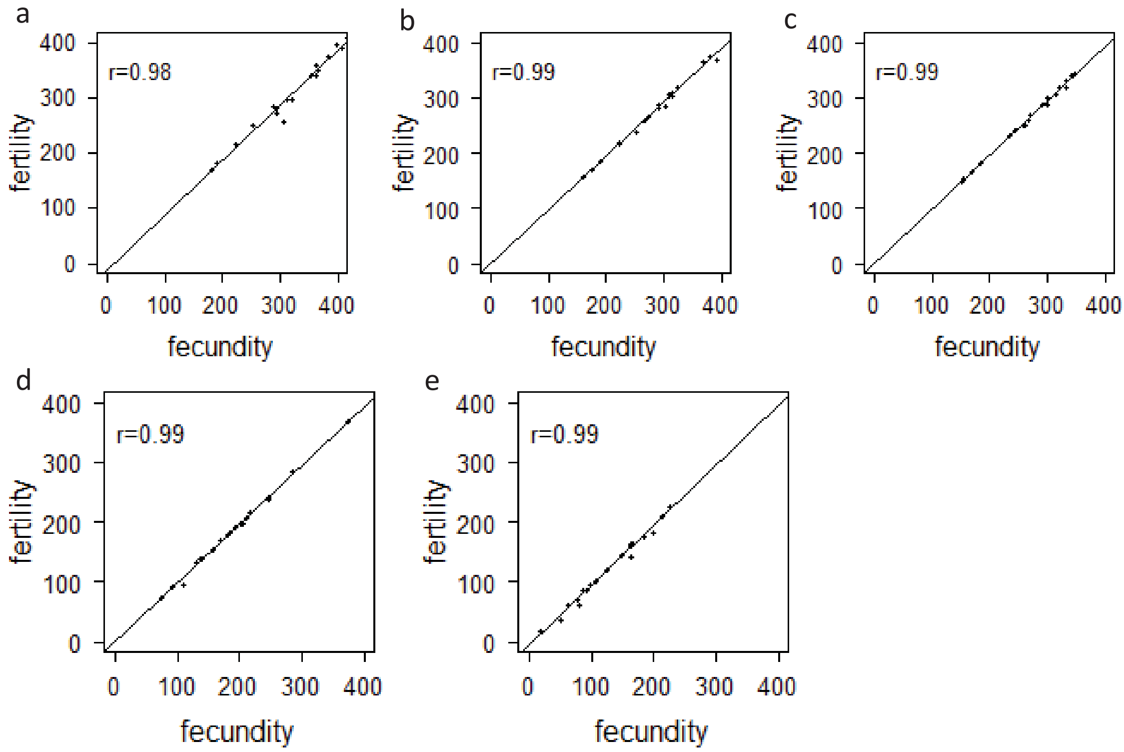

Figure 2. Correlation between fecundity and fertility in *Ephestia kuehniella* females that were reared at population densities of 50, 100, 200, 400 & 800 (a-e).

Discussion

Population density and associated food stress have a large impact on developmental rate in the gypsy moth *Porthetria dispar* (Leonard, 1968), growth rate in autumnal moth *Epirrita autumnata* (Tammaru et al., 2000), survival in mosquito *Aedes aegypti* (Macia, 2009), body size in *Loxostege sticticalis* moths (Kong et al., 2011), and adult reproductive output in scarab beetles *Aphodius ater* (Hirschberger, 1999) which in some instances may be sex-specific (e.g. Fantinou et al., 2008).

In the present study it was found that population density had a negative effect on life-history traits and reproductive output of *E. kuehniella* (Tables 1, 2 & Figure 1), because at higher population densities food was exhausted quickly. Prolonged larval developmental period may be an outcome developed by food-stressed larvae to increase the time available to find more nutrients and gain the minimum weight required for successful emergence (Bauerfeind and Fischer, 2005; Hooper et al., 2003). Due to increasingly delayed development or cannibalism (Savoldelli, 2006) or microbial infestation due to food contamination with dead larvae and insect faeces (Stone and Sims, 1991) the lowest percent pupation occurred at the highest population density (800). Both males and females that developed at highest population density (800) had lower pupal mass (Table 2) indicating that larvae cannot compensate for the effects of food shortage despite prolonging developmental period. It can also be suggested that both developmental period and body size are important life-history traits in *E. kuehniella*.

Both the fecundity and fertility of resultant females decreased with increased larval rearing density (Figure 1) and a similar pattern has been reported in the beetle *Dacne picta* (Sato et al., 2004), cabbage moth *Mamestra brassicae* (Kazimirova, 1996) and pink stalk borer *Sesamia nonagrioides* (Fantinou et al., 2008). This can probably be attributed to limitations in food resources. Food stress also delays and reduces the egg production and maturation process due to associated changes in neuroendocrine responses (e.g. Slansky, 1980) and release of egg maturation chemicals (Watt, 1986). In *E. kuehniella* although fecundity at the lowest population density (50) was the highest there was no significant difference in fertility (the realised reproductive output) between larval rearing densities of 50 to 200. When the population density reached 400, both fecundity and fertility decreased significantly (Figure 1). This indicates that the total amount of resources available to larva determines female fecundity but due to physiological or behavioural constraints or male ejaculates that affect fertility, females at lowest population density (50) do not have greater realized reproductive output (fertility) resulting in relatively low correlation between fecundity and fertility at lowest population density (50) compared to others (Figure 2).

The results of the present study suggest that the optimal rearing density for this species should be 200 larvae per 50 g of diet as this result in more adults without significant loss of fecundity. Although 100 larvae per 50 g of diet has been previously recommended (Xu et al., 2007) the density treatments in that study were too widely spaced and the density of 200 larvae was not tested.

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STATEMENT OF CONTRIBUTION TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Santhi Priya Bhavanam

Name/Title of Principal Supervisor: Steve Trewick

Name of Published Research Output and full reference:

Bhavanam, S., Wang, Q., and He, X. (2012). Effect of nutritional stress and larval crowding on survival, development and reproductive output of Mediterranean flour moth, Ephestia kuehniella Zeller. New Zealand Plant Protection 65, 138-141.

In which Chapter is the Published Work: Chapter 2

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate 90%

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  I conducted the experiment and collected data. Zhao and I did data analysis. I wrote the initial draft of the manuscript and subsequently edited by Qiao, Zhao and me taking referees comments into consideration.

Candidate's Signature

11-01-16
Date

Principal Supervisor's signature

11 January 2016
Date

CHAPTER 3

Effects of nutritional limitation on female phenotype in relation to fecundity

Abstract

Allocation of resources to reproduction varies with nutrient availability. Theory predicts that under poor conditions, females should increase their reproductive fitness by investing more in traits that affect offspring fitness. Although many studies have focused on trade-offs between life-history traits and reproduction, the effects of female morphology on dispersal, reproduction and oviposition have received less attention. Here, I investigated the effects of larval nutrient limitation on adult morphology using female Mediterranean flour moths, *Ephestia kuehniella* that were subjected to nutrient stress through manipulation of population densities*.* Females that emerged from higher density larval population had reduced adult body mass and sizes of head, thorax, forewing and abdominal depth. Food stress significantly altered the allometric relationship between abdominal depth and body mass and food stressed females had bigger abdomens relative to their mass. Abdomen size is likely linked to fecundity as it influences egg number. This indicates changes in fecundity and females invest more in reproduction to reduce the negative effects of food shortage on reproduction. Ovipositor length remained unchanged among treatments, indicating functional constraints on some reproductive traits. This study suggests that traits related to female reproductive fitness are conserved and when resources are limited, trade-offs occur with females diverting more resources in traits that are related to offspring. These results are likely relevant to many semelparous species that depend on larval resources for reproduction.

Introduction

Reproductive fitness is maintained by the production of viable reproductive offspring. The amount of resources allocated to reproduction effects not only the current reproductive cycle but also has the potential to effect the success of future generation mediated through oviposition decisions and egg provisioning changes (Mousseau and Fox, 1998). For females the pattern of resource allocation may vary depending on reproductive mode (i.e. semelparity and iteroparity), mate availability, habitat stability and available nutrient reserves (Boggs, 1981). Moreover, theory predicts that in poor quality environments females should increase their investment in traits that can influence offspring success. Therefore, increased investment in one trait may result in trade-offs with others and females may develop a reproductive strategy that suits the local conditions (Pianka and Parker, 1975) in response to natural selection (Pianka, 1976).

Nutrient limitation is one of several critical environmental factors that individuals face during their life. Low nutrient availability during juvenile stages reduces growth rates and prolongs development resulting in maturation at small size with limited resource reserves. Such adults have lower reproductive success (Roff 2002). As a response to deteriorating habitat quality, individuals in some species allocate more resources to dispersal so better quality environments might be discovered. This has been documented in wing-dimorphic and long-lived wing monomorphic insects where adult survival takes priority over reproduction but reproductive fitness is increased later in life (e.g. Pellegroms et al., 2009; Saastamoinen et al., 2010). However, as per oogenesis-flight syndrome theory (Johnson, 1969), dispersal and reproduction are negatively correlated with each other and increased allocation of fixed energy reserves to flight decreases the amount available for reproduction (Jervis et al., 2005). As a result adults have lower fecundity and offspring fitness. Moreover, migration that occurs prior to reproduction delays egg maturation and extends the pre-oviposition period (for a review see, Zera and Denno, 1997; Zera and Harshman, 2001) leaving adults less time to reproduce. Furthermore, food stress during juvenile stage shortens adult life-span in Lepidoptera and it is predicted that in short-lived species, food-stressed females will invest more in reproduction.

In insects, resources allocated to abdomen growth are dependent on overall body size (e.g. Kivela et al., 2012) resulting in a positive correlation between body mass and abdomen mass (Wickman and Karlsson, 1989). So, a decrease in body size is associated with reduced abdomen size and a decrease in fecundity (e.g. Fantinou et al., 2008). Moreover, females of low reproductive potential have lower mating success because males prefer to mate with larger females (Bonduriansky, 2001). Numerous examples show that food shortage (nutrient stress) during the juvenile stage lowers female fecundity and mating frequency (e.g. Bhavanam et al., 2012; Tigreros et al., 2013; Tripathi and Singh, 1990) but the few studies that observed how allocation to reproduction changes with environment have found contrasting results (e.g. Boggs and Freeman, 2005; Stevens et al., 2000).

Females may also reduce negative developmental constraints through behavioural compensation (Berger et al., 2012). One way that females can influence offspring phenotype and fitness is through oviposition behaviour (Mousseau and Fox, 1998). When and where eggs are deposited determines the pre and post-hatching environment of offspring including the level of competition (e.g. Kruger and Mills, 1990), predation risk (e.g. Labouche and Bernasconi, 2013), pathogen attack risk (e.g. Gerard, 1989), resource quality (e.g. Murray, 1985) and climatic variation (Masaki, 1986). All such "maternal effects" contribute to offspring survival and in turn female reproductive fitness (Mousseau and Fox, 1998). The ovipositor is the organ used to lay eggs (Chapman, 1998) and the length of the ovipositor determines the depth of egg deposition in the brood media (Bradford et al., 1993). As a result ovipositor length differs, in relation to a substrate type (e.g. Joy and Crespi, 2007), egg traits (e.g. Yanagi and Tuda, 2010) and larval environment (Masaki, 1986) indicating that it is subject to natural selection.

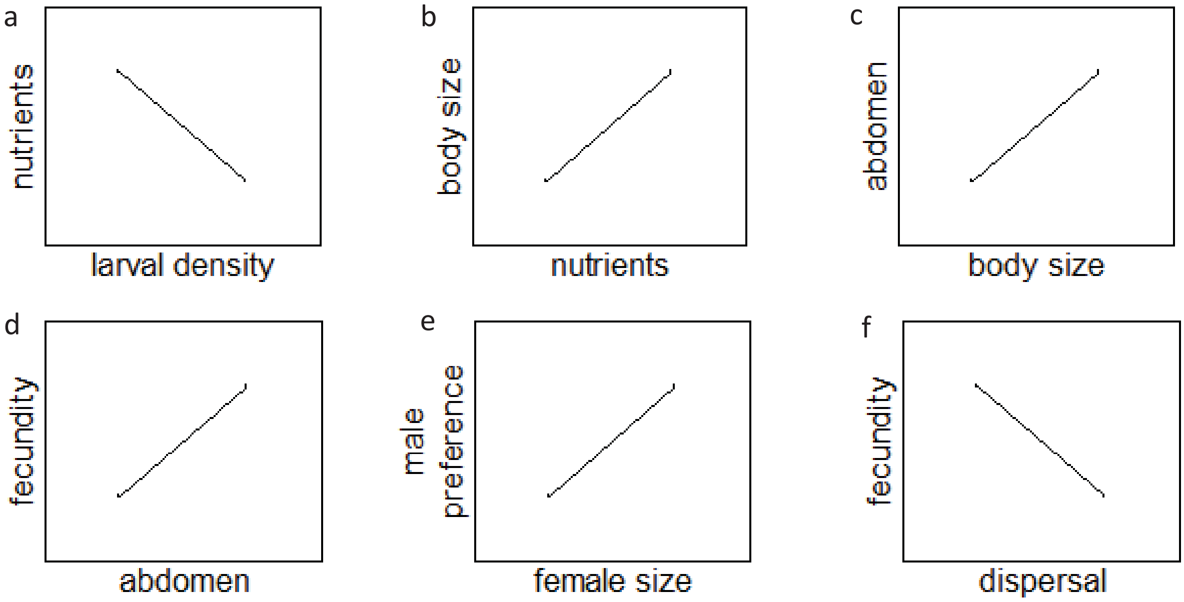

Figure 1. Schematic illustration of the changes associated with increased population density on adult female, focusing on its effects on dispersal and reproduction. In a constant food source, nutrient availability and population density are inversely proportional (a). Decrease in amount of available nutrients has a negative effect on body size and in turn abdomen and fecundity as the later three are positively related (b-d). Small females due to lower fecundity are less preferred by males (e). Changing allocation strategies and shifting wing morphology and flight in response to food stress can have a detrimental effect on fecundity (Flight-oogenesis syndrome) (f).

The interactions between resource availability, female phenotype and fecundity are complex (Figure 1). In holometabolous insects during metamorphosis fixed larval resources are partitioned and allocated to soma and non-soma in a hierarchical manner (Jervis et al., 2005; Jervis et al., 2008). Soma includes the head, thorax and wings while reproductive tissue and gametes situated in the abdomen constitute non-soma. Investment in somatic structures influences dispersal ability and survival and the latter used for reproduction (Jervis et al., 2005; Jervis et al., 2008). In this study, I altered resource availability through the manipulation of population density and observed how resulting nutrient limitation affected expression of traits related to dispersal, fecundity and oviposition. It was hypothesised that resource-limited females would increase their investment in reproduction (abdominal depth and ovipositor). To observe these effects I compared the response of different traits and the allometric relationship between body mass and morphological traits of female *E. kuehniella* from different population densities. It was further hypothesised that resource-limited females would not alter their investment in flight because after initial oviposition the ratio of wing length to body mass increases. I also observed the mating and daily oviposition pattern of females because delayed egg maturation or pre-reproductive period is related to flight in many insects (e.g. Colvin and Gatehouse, 1993; Hill and Gatehouse, 1992).

Materials and methods

Study species

Ephestia kuehniella, Mediterranean flour moth (Lepidoptera: Pyralidae) is a stored product pest. Larvae feed on flour and animal products (Rees and Rangsi, 2004). Nutrient limitation and larval crowding of juveniles affects their development, survival and pupal mass (chapter 2). Adults do not feed (Norris, 1934) and have a short life-span so all nutrients that are required for growth and reproduction must be obtained during the larval stage. Therefore, it can be predicted that any change in the environment experienced by juveniles will affect the adult phenotype. Female abdominal thickness (depth) is known to be positively correlated with lifetime fecundity in this species (Xu and Wang, 2010) and that males prefer large females as mating partners (Xu and Wang, 2009a). An adult female lays up to 300 eggs but this number decreases with increased population density in which she develops (chapter 2).

Laboratory stock cultures of *E. kuehniella* were maintained in plastic containers (8 cm in diameter × 10 cm in high). In each container, 200 larvae were reared on 50 g of a prepared dietary medium comprising wholemeal wheat flour (43.5%), maize meal (43.5%), brewer's yeast (3%) and glycerine (10%) (Lima-Filho et al., 2001) at 25 ± 1˚C and 60 ± 10% relative humidity with a photoperiod of 14:10 h (light:dark) cycle. Eggs less than 24 h old were harvested from stock culture mated females and used to establish four experimental population densities: 100, 200, 400 and 800. Each was provided with 50 g of diet and maintained in the same conditions above. Mature pupae were collected and placed individually in separate glass vials (2 cm in diameter × 7.5 cm high). Adults that emerged in the glass vials were sexed and used for morphometric study.

From each experimental population density of *E. kuehniella*, 30 adult females less than 12 h old were selected randomly, anesthetized with CO_2 and the adult body mass of each recorded using an electronic dual range balance (Mettler AE100, Mettler-Toledo Inc., Switzerland) with a precision of 0.1 mg. The abdominal depth of each adult was measured as the distance between dorsal and ventral surfaces of the 4th abdominal segment. Adults were then killed by freezing at —20˚C for 24 h before further measurements were taken. The forewing length from the point of articulation to the distal end of the forewing and the head and thorax width at their widest points were measured (Figure 2). Subsequently, females were dissected to determine the length of the ovipositor between the top of ovipositor lobes and the bottom of posterior apophyses. All morphological traits were measured using digital images captured by a USB 2.0 Video/Audio Grabber (Lindy Electronics Ltd, UK) with Universal Desktop Ruler software (AVPSoft, USA).

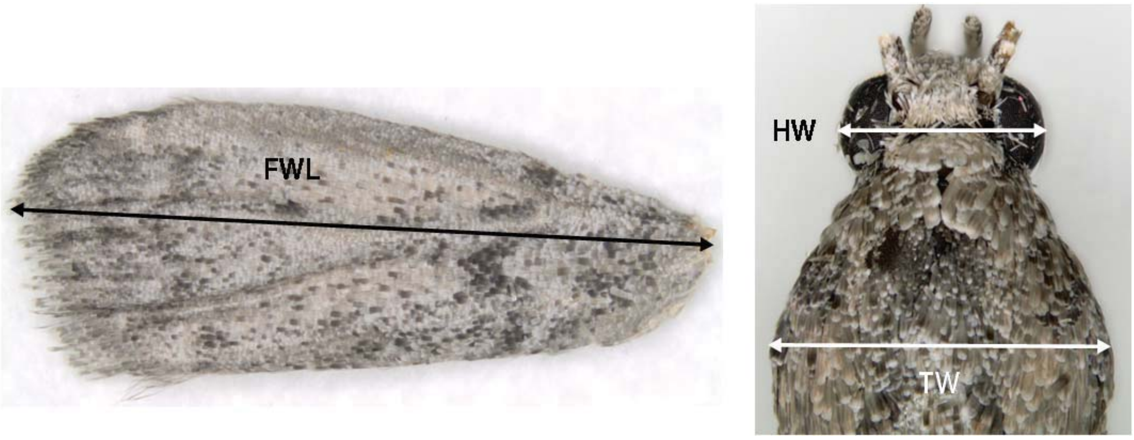

Figure 2. External morphological measures made on adult *Ephestia kuehniella*. FWL, HW and TW represent forewing length, head and thorax widths, respectively.

Daily oviposition pattern

To test whether population density had an effect on female daily oviposition pattern, newly emerged virgin males and females 20 randomly selected from each density were paired in separate plastic containers for their lifetime (8 cm in diameter × 10 cm in high). Eggs were collected daily from each pair and maintained separately until the female died. Harvested eggs were placed in Petri dishes for incubation. After three days of incubation, eggs were examined for fertility under a dissecting microscope (Olympus SZ III, Japan) for the presence of larval heads (black dots). Daily and lifetime fecundity and fertility were counted for each female and length of oviposition period recorded.

Effect of depth on

Egg deposition depth is a function of ovipositor length, and a change in ovipositor length is associated with a change in the depth at which eggs were deposited. In the present study, as the allometric relationship between ovipositor length and body mass was not significantly affected by population density and body mass (Table 1, 2, & Figure 4d), I examined if there was relation between egg predation and juvenile survival and egg laying depth.

Neonate larval survival

To determine whether depth of the egg deposition had an effect on juvenile survival, four treatments were established by placing 10 eggs each at four different depths: 0mm (surface), 1mm deep, 2mm deep and 3mm deep in separate glass vials that were filled with a constant amount of 2.5 g diet. For the first treatment, the eggs were spread on the surface of the diet. To place the eggs at 1mm deep, the weight of diet to 1mm depth in the glass vial was measured and removed. Eggs were placed on the top of the remaining diet and then covered with the removed diet thus making up the weight to 2.5 g. A similar procedure was followed for 2 and 3mm depths. For each treatment, there were 45 replicates. After the first larval moult, the number of surviving larvae was counted and neonate larval survival (%) was calculated as the number of larvae survived/number of eggs placed multiplied by 100.

Egg predation

To determine whether burial of *E. kuehniella* eggs influenced the rate of their predation an experiment used the polyphagous hemipteran *Orius vicinus* that predates *E. kuehniella* and other invertebrates (Lariviere and Wearing, 1994). Nymphs of *O. vicinus* were reared on the frozen eggs of *E. kuehniella* in a glass vial covered with a ventilated lid. High relative humidity was obtained using moistened Anchor blue seed germination blotter (Anchor Paper Co, USA) or a moistened cotton ball placed at the bottom of the glass vial and covered by a filter paper (Castane et al., 2014). Fresh eggs as feed were placed on the blotter/filter paper and replaced every second day until adult emergence.

One day old eggs were collected from 15 mated females and placed in separate Petri dishes. Eggs from each mother were allocated to all four treatments: 1. exposed eggs; 2. eggs buried to 1mm depth in dietary media; 3. to 2mm depth in dietary media; and 4. to 3mm depth in dietary media. For each treatment, 10 eggs were placed in a glass vial and one *O. vicinus* adult was released for feeding. After 24 h, the predator was removed and the remaining eggs incubated. On the 4th day of development, the eggs with visible black heads were counted with the aid of dissecting microscope (Olympus SZ III, Japan). Egg predation (%) was calculated as the number of viable eggs/ number of eggs placed for each treatment. There were fifteen replicates for each treatment.

Statistics

All analysis were performed using SAS version 9.3. The Shapiro-Wilk test was used to test the normality of the data. Data for adult body mass, forewing and ovipositor length, abdominal depth, head and thorax width, daily oviposition pattern, neonate larval survival (%) and egg predation (%) were found to be normally distributed and analysed using ANOVA followed by Tukey's HSD test for multiple comparisons. The relationship between the morphometric traits and body mass were analysed using linear regression. An ANCOVA with body mass as a covariate was performed to compare the slopes of the linear regressions. The rejection level was set at $P < 0.05$.

Results

Adults that emerged from the lowest population density (100) were significantly heavier than those that emerged from other population densities ($F_{3,114} = 26.76$, $P < 0.0001$) (Table 1). Population density had no significant effect on the length of the ovipositor ($F_{3,96} = 2.05$, $P = 0.112$). However, forewing length ($F_{3,111} = 5.6$, $P = 0.001$), head width ($F_{3,111} = 7.54$, $P = 0.001$), thorax width ($F_{3,113} = 12.2$, $P < 0.0001$) and abdominal depth ($F_{3,113} = 8.64$, $P < 0.0001$) were significantly greater at population densities of 100 and 200 than at a population density of 800 (Table 1).

Table 1. Mean (± SE) sizes (mm) of various morphological traits of female *Ephestia kuehniella* selected at random after culture at a range of densities. Means followed by the same letters in each row are not significantly different ($P > 0.05$).

Morphological trait	100	200	400	800
Adult body mass	21.74 ± 0.44 a	20.49 ± 0.47 b	19.43 ± 0.34 b	16.45 ± 0.39 c
Abdominal depth	1.80 ± 0.03 a	1.78 ± 0.02 a	1.69 ± 0.02 ab	1.62 ± 0.06 b
Fore wing length	8.85 ± 0.07 a	8.53 ± 0.08 ab	8.50 ± 0.08 ab	8.34 ± 0.12 b
Head width	1.25 ± 0.01 a	1.25 ± 0.01 a	1.22 ± 0.01 a	1.19 ± 0.01 b
Thorax width	2.35 ± 0.03 a	2.25 ± 0.03 ab	2.19 ± 0.02 bc	2.12 ± 0.03 c
Ovipositor length	2.45 ± 0.03 a	2.43 ± 0.04 a	2.40 ± 0.04 a	2.30 ± 0.08 a

Within treatments many morphological traits were positively correlated with body mass (Table 2, Figure 3). In keeping with observations of ovipositor length among treatments (Table 1), there was no significant relationship between female weight and ovipositor length within most treatments (200, 400 & 800) (Table 2) and the slopes of the regression lines were homogeneous ($F_{3,92} = 0.67$, $P = 0.575$ for ovipositor length) (Figure 3d). There was a significant positive relationship between body mass and abdominal depth except for the population density of 400 (Table 2). ANCOVA indicated that the slope of the female abdominal depth at the population density of 800 was steeper than at other population densities ($F_{3,93} = 4.25$, $P = 0.007$) (Figure 3b). For other morphological traits, a significant positive relationship between body mass and trait size was also detected at various population densities (Table 2) and the slopes of the linear regressions did not differ significantly between population densities for forewing length ($F_{3,107} = 0.53$, $P = 0.663$), head width ($F_{3,107} = 0.23$, $P = 0.873$) and thorax width ($F_{3,107} = 0.34$, $P = 0.787$) (Figure 3a, c, & e).

Table 2. The relationship between body mass (x, mg) and morphological trait size (y, mm) in width and length) of *Ephestia kuehniella* female reared at different population densities. Regression equations are given as y = a + bx, where y is the dependent variable (morphological trait size), x is body mass, a is intercept and b is slope. All values were log-transformed. Significant *P* values are indicated in bold.

Morphological trait	Population density	Linear regression	R^2	*F*	*P*
Fore wing length	100	y = 0.71 + 0.17 x	0.215	1,28=7.69	**0.010**
	200	y = 0.60 + 0.26 x	0.390	1,28=17.93	**0.000**
	400	y = 0.60 + 0.26 x	0.277	1,27=10.35	**0.003**
	800	y = 0.72 + 0.16 x	0.139	1,24=3.87	0.061
Head width	100	y = —0.10 + 0.16 x	0.268	1,28=10.24	**0.003**
	200	y = —0.16 + 0.19 x	0.206	1,28=7.24	**0.011**
	400	y = —0.06 + 0.12 x	0.165	1,27=5.32	**0.030**
	800	y = —0.11 + 0.16 x	0.334	1,24=12.05	**0.002**
Thorax width	100	y = —0.00 + 0.29 x	0.324	1,28=13.43	**0.001**
	200	y = 0.08 + 0.21 x	0.172	1,28=5.80	**0.023**
	400	y = 0.12 + 0.17 x	0.109	1,27=3.30	0.081
	800	y = 0.00 + 0.27 x	0.311	1,24=10.87	**0.003**
Abdominal depth	100	y = —0.38 + 0.47 x	0.313	1,27=12.31	**0.002**
	200	y = —0.07 + 0.25 x	0.185	1,27=6.12	**0.020**
	400	y = —0.03 + 0.19 x	0.051	1,25=1.35	0.257
	800	y = —1.22 + 1.16 x	0.463	1,14=12.09	**0.004**
Ovipositor length	100	y = 0.06 + 0.25 x	0.237	1,25=7.77	**0.010**
	200	y = 0.25 + 0.10 x	0.022	1,25=0.56	0.463
	400	y = 0.53 – 0.12 x	0.017	1,22=0.39	0.539
	800	y = 0.12 + 0.20 x	0.048	1,20=1.01	0.327

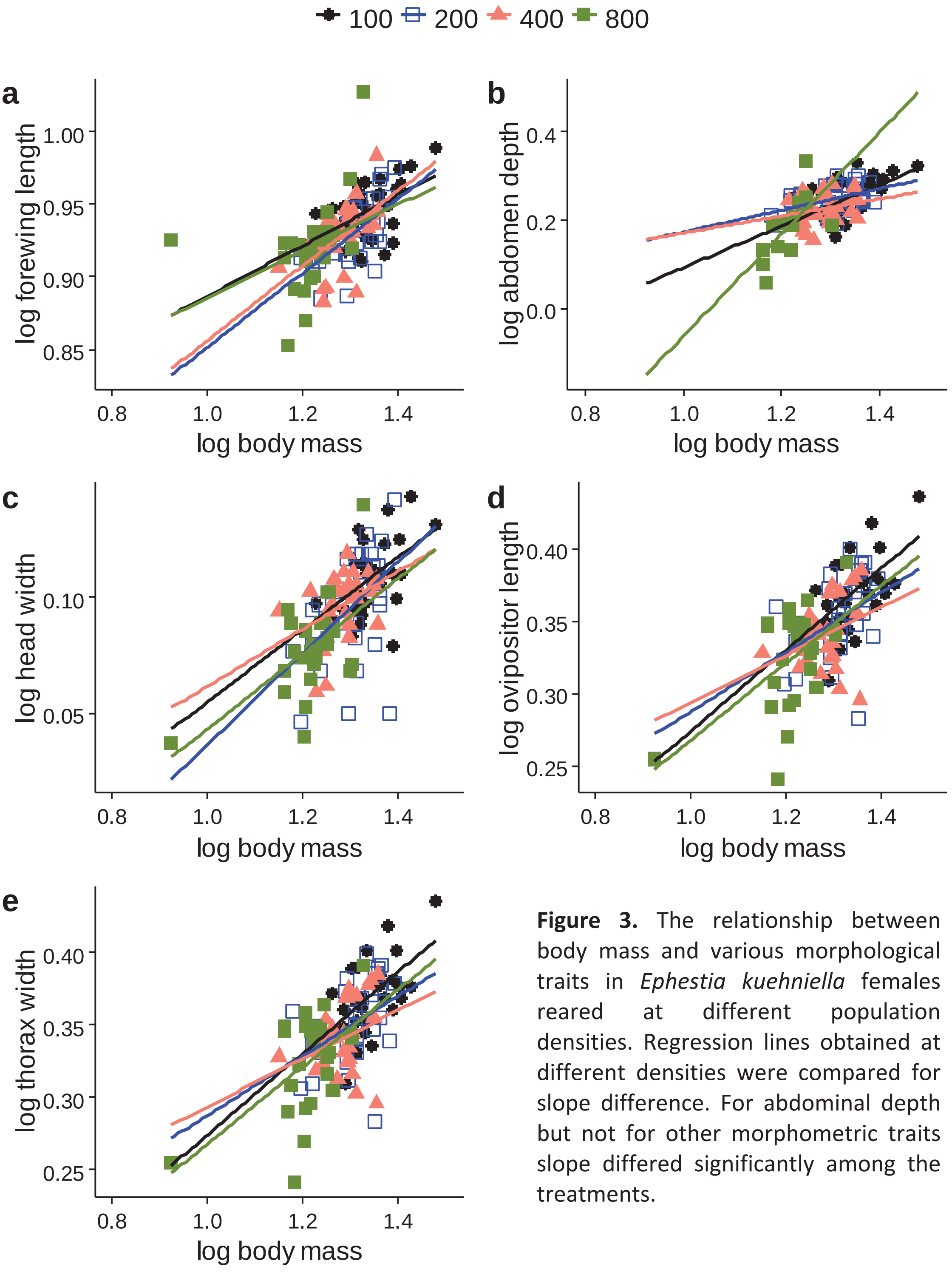

Figure 3. The relationship between body mass and various morphological traits in *Ephestia kuehniella* females reared at different population densities. Regression lines obtained at different densities were compared for slope difference. For abdominal depth but not for other morphometric traits slope differed significantly among the treatments.

In the other set of females that were allowed to mate and lay eggs, it was found that irrespective of the rearing density, the oviposition period lasted for a maximum of 6 days. The daily oviposition pattern was not constant with > 80% of fecundity realized in the first three oviposition scotophases in females from all population densities (Figure 4). In the first oviposition scotophase, the numbers of eggs laid by females from 100, 200 & 400 densities were significantly higher than in the second and third oviposition scotophases and both were significantly higher than fourth, fifth and sixth scotophases (100: $F_{5,80} = 33.45$, $P < 0.0001$; 200: $F_{5,88} = 23.30$, $P < 0.0001$; 400: $F_{5,79} = 12.47$, $P < 0.0001$). At a population density of 800, there was no significant difference in fecundity until third oviposition scotophase which decreased thereafter ($F_{4,70} = 7.52$, $P < 0.0001$). Across the treatments, in the first oviposition scotophase, females from the higher population densities (400 & 800) laid significantly fewer eggs than those from lower population densities ($F_{3,69} = 17.00$, $P < 0.0001$). In the remaining oviposition scotophases, no difference in fecundity was observed among the treatments (second: $F_{3,53} = 1.90$, $P = 0.142$; third: $F_{3,67} = 2.37$, $P = 0.079$; fourth: $F_{3,60} = 1.14$, $P = 0.340$; fifth: $F_{3,50} = 0.495$, $P = 0.687$; sixth: $F_{3,18} = 1.13$, $P = 0.344$) (Figure 4).

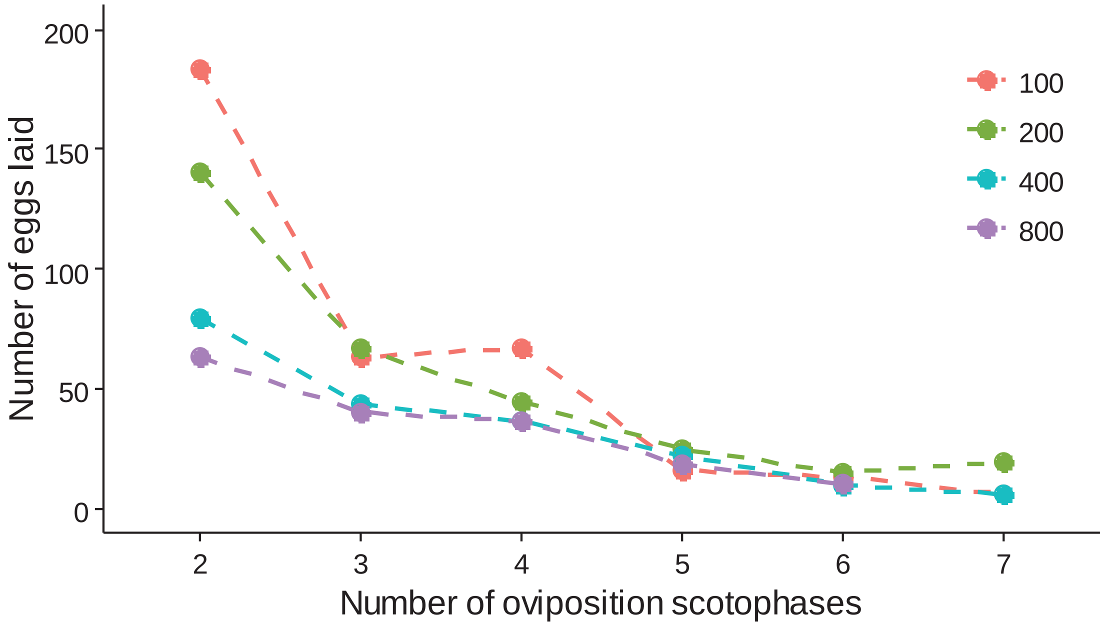

Figure 4. Mean numbers of eggs laid in six oviposition scotophases by female *Ephestia kuehniella* reared at different population densities. Multiple comparisons (Tukey's HSD test) showed that females emerged from population densities of 100, 200 & 400 except population density of 800 deposited more number of eggs in the first oviposition scotophase compared to other scotophases, however, in the same scotophase females emerged from population densities of 100 and 200 had laid significantly more eggs than those from higher population densities (400 & 800) ($P < 0.05$).

The percent of surviving larvae was not significantly affected by egg deposition depth ($F_{3,56} = 1.75$, $P = 0.168$) (Figure 5a). The predation rate was significantly higher on eggs placed at the surface than that at 1, 2 & 3 mm depth in diet ($F_{3,50} = 8.73$, $P < 0.0001$) (Figure 5b) and resulted in reduced offspring survival.

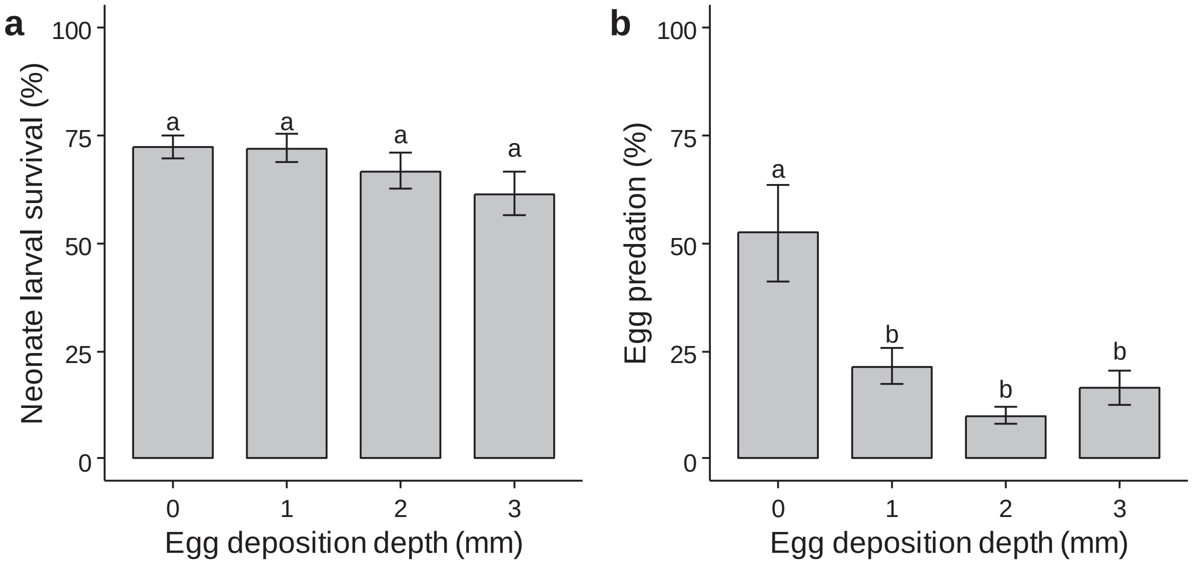

Figure 5. Effect of egg deposition depth on *Ephestia kuehniella* neonate larval survival (a) and egg predation (b). Different letters above each bar indicate significant difference ($P < 0.05$).

Discussion

Increased population density at the juvenile stage exerts its influence on adult phenotype through a reduction in nutrient availability. As a result adults emerging in crowded conditions are small and have smaller body parts compared to those in less crowded conditions (e.g. Boggs and Freeman, 2005; Dmitriew et al., 2009). Abdominal mass and thickness are affected by food shortage (e.g. Fantinou et al., 2008; Hooper et al., 2003; Sato et al., 2004; Watt, 1986) and decrease due to lower fecundity or presence of few mature eggs. Decrease in food quantity changes resource allocation patterns and as a result the allometric relationship between body mass and different morphological traits differs. For example, females of some species may increase investment in the abdomen (e.g. Jannot et al., 2007) while others shift their wing-morphology (see Angelo and Slansky, 1984).

In the present study, I found that at a higher population density (800) the values for morphological traits such as forewing length, head and thorax width, and abdominal depth decreased (Table 1). As predicted, the slope of the allomteric relationship between body mass and abdominal depth was steeper at a population density of 800 compared to other densities (Figure 3b). However, the relationship between forewing length and widths of head and thorax did not differ among the treatments (Figure 3a, c & e). Population density had no effect on the mean ovipositor length (Table 1). Moreover, there was no significant difference in the allometric relationship between body mass and ovipositor length of females reared at different population densities (Figure 3d). The increased investment of resources into the abdomen by food-stressed *E. kuehniella* females suggests that resource allocation occurs in a manner that suits the adult life-history strategy and the reproductive success is increased.

Adult *E. kuehniella* do not feed (Norris, 1934) and are short-lived. As a result females use resources acquired during the larval stage for egg production (Gullan and Cranston, 2009) and have a complete egg load that matures within 48 h of adult emergence (Calvert and Corbert, 1973). Females mate when they are one day old (Xu et al., 2008) and those with deeper abdomens are most successful in obtaining mating (Xu and Wang, 2010) as abdomen size is positively correlated with fecundity (Xu and Wang, 2010). Maximizing egg production at emergence by food-stressed females may be beneficial as it helps to overcome the disadvantages of being small and enhances the mating success. This is especially important in species such as *E. kuehniella* where adults have short lifespans. This study shows that female body dimensions are reduced by food shortage arising from a high population density and indicates that specific trait reductions occur in a manner that has the least impact on reproduction.

Despite this obvious benefit of increased offspring number due to increased investment in the abdomen, stressed females maintained their allocation to the ovipositor. In species that have a single reproductive opening, male and female genitalia co-evolve for successful mating and insemination. For example, Althoff (2014) demonstrated that in yucca moths, changes in oviposition strategy resulted in changes in ovipositor morphology and in turn the male intromittent organ. However, this explanation can be eliminated for *E. kuehniella* as female have two separate reproductive openings, vulva for mating and ovipore for oviposition (Drummond, 1984). Therefore, we predicted that ovipositor length may be related to oviposition.

It has been proposed eggs placed deeply into the substrate may not have access to food and other resources while those laid superficially are exposed to predation and desiccation (Masaki, 1986). Both contribute to greater offspring mortality. For example, in fig wasps, larval survival rate of deeply inserted eggs was low (Murray, 1985). Likewise, in crickets, egg laying depth and hatchling survival were inversely related (Bradford et al., 1993). In the present study, I found no significant negative effect of depth on the number of surviving larvae in the absence of predators (Figure 5a). This may be because of congenial environmental conditions and abundant food supply at the site of egg hatching.

I also show that eggs placed on the surface are subject to more predation that contributed to low larval survival in this treatment compared to other treatments (Figure 5b). This partly supports Masaki's hypothesis (1986). In *E. kuehniella*, the ovipositor consists of a pair anterior and posterior gonapophyses (Norris and Richards, 1932). During oviposition, females bend their abdomen in such a way that the direction of the ovipositor shifts from posterior to ventral (Chapman, 1998). The ovipositor is then pushed into the substrate by the synchronous movements of the two pairs of cross-connected gonapophyses (Scudder, 1971). Females with a short ovipositor drop the eggs superficially on the substrate and that reduces larval survival and so female reproductive fitness. Therefore, it appears that optimal ovipositor length has evolved through a mixture of abiotic and biotic environmental impacts on the offspring.

The lack of any effect of nutrition limitation and larval crowding on wing morphology at higher density could suggest a trade-off. Females at this density had deeper abdomens relative to their body mass, which indicates that they carry a relatively large initial egg load as abdominal depth (thickness) is a reliable indicator of female fecundity (Xu and Wang, 2010). This attribute does not favour flight in Lepidoptera (Sattler, 1991) and other insects (Evans, 2003), and flight and egg production compete for similar resources, especially protein (Wheeler, 1996). The observed increase in abdominal allocation is expected to have decreased the resources available for flight in *E. kuehniella*, inferred for some insect species (Wheeler, 1996).

My previous observations showed decreased resources associated with crowding during the larval stage delayed adult emergence by 5 days compared to optimal conditions (chapter 2). Adult flight (dispersal behaviour) is a response to food availability during the juvenile stage (Dingle, 1972). During development of flight capability, no ovarian development occurs, and as a result the pre-oviposition period increases (e.g. Colvin and Gatehouse, 1993; Hill and Gatehouse, 1992; Wilson and Gatehouse, 1992). When these factors are coupled together, adult reproduction is considerably delayed. *Ephestia kuehniella* females obtain their first mating when they are one day old and complete > 80% of mating and oviposition activities within 4 days of emergence (Figure 4). Therefore, late onset of reproductive capability as a result of developmental flight capability would be expected to reduce the mating chances because of lack of mates, with resulting death of same female before mating. This study suggests that a flight response would not be beneficial to stressed *E. kuehniella* females.

The pattern of mating and oviposition favours flight late in life (Figure 4). In the African armyworm *Spodoptera exempta*, after initial mating and oviposition the body mass of females decreases and in turn the ratio between wing length and body mass increases (Aidley and Lubega, 1979). As a result, stressed females of *E. kuehniella* can move to new habitats without additional costs of reproduction. Moreover, females can reduce the risk of not mating as the sperm stored from a single ejaculate is sufficient to fertilize the entire egg load (Xu and Wang, 2009a). Although 50% of eggs are deposited in 1–2 days in the current habitat, these females still have the remaining 50% of their eggs that could be laid after flight in a new habitat to potentially increase their reproductive fitness.

In summary, population density in *E. kuehniella* reduces the mean sizes of various morphological parts except the ovipositor. Metamorphosis provides an opportunity to alter resource allocation patterns and buffer against environmental constraints. The lack of a treatment effect on wing morphology is best explained with reference to the short adult lifespan of this species where flight prior to reproduction is not beneficial. The allometric relationship between body mass and abdominal depth differs, which may be a local reproductive strategy developed by females at the highest population density (800) to maximize their attractiveness and reproductive output. The ovipositor length remains unaffected by rearing density. As offspring survival is affected by egg deposition depth and stressed females may suffer more if they decrease their ovipositor length as that may result in zero reproductive fitness. This study suggests that females strategically adjust their allocation pattern based on their life-history strategy so that they have greater reproductive fitness.

CHAPTER 4

Effects of larval crowding and nutrient limitation on male phenotype, reproductive investment and strategy in *Ephestia kuehniella* (Insecta: Lepidoptera)

Abstract

Food shortage during the juvenile stage affects the adult morphology and life-history traits that have consequences on male reproductive success. Males therefore adjust their resource allocation patterns and find a balance between ecological and sexual traits that maximises their reproductive fitness. *Ephestia kuehniella* adult males were reared at different population densities (100, 200, 400 & 800 larvae) to investigate phenotypic shifts under controlled conditions. Sizes of different ecological morphometric traits such as forewing, head and thorax and adult longevity and sexual traits such as valva and aedeagus, sperm number, mating frequency were compared. Larval rearing treatment had a substantial effect on the adult phenotype. Males that emerged from higher population density (800) had lower mass and small forewings, head and thorax. This suggests that adults cannot completely compensate for food shortage. It was also apparent that the allometric relationship between body mass and forewing length changed and these males had relatively longer wings. This may be an arrangement that enhances dispersal to cope with food shortage at higher densities. This may also aid in other foraging behaviour such as mate-searching, as at higher densities male reproductive success was expected to depend on male agility and the ability to find females. Males developed at a population density of 800 achieved similar mating frequency as males at other densities. This was despite them producing fewer eupyrene but not apyrene sperm and having a shorter adult lifespan. These results indicate that by mating more frequently and keeping the apyrene numbers similar, males increase their reproductive success at sperm competition seen at higher densities. Food stress associated with high population density did not affect valva

and aedeagus size, which indicates that these traits may be insensitive to external environmental changes because they incur fitness costs to males. Our results suggest that males are sensitive to the larval environment and develop a reproductive strategy that suits local conditions that help increase net reproductive fitness.

Introduction

Organisms face constantly changing biotic and abiotic conditions that could reduce individual fitness (Koehn and Bayne, 1989; Rose, 1991). Generally, individuals detect environmental changes and alter their resource allocation pattern, trade-off in behaviour and resources in a manner that maximizes their lifetime fitness (Monaghan, 2008). Although a critical component of evolutionary ecology, we still know little about the possible strategies that support this process (Boggs, 2009).

In insects, variations in population size mediated through larval crowding and nutrient limitation during development have a negative effect: individuals developed under poor conditions typically have reduced body mass, growth rates, life span and reproductive success compared to individuals developed under more favourable conditions (for example, Fantinou et al., 2008; Macia, 2009; Miller and Thomas, 1958). To mitigate these negative effects, some species increase energy reserves (e.g. cockroach *Nauphoeta cinerea* Barrett et al., 2009) and metabolic rate (e.g. butterfly *Bicyclus anynanaes* Pijpe et al., 2008) that increases the capacity of individuals to overcome stress. However, sometimes individuals may opt to move away from bad conditions. Consequently, changes to morphology, body composition and behaviour of adults that relate to dispersal are initiated during metamorphosis. This type of development has been reported in both wing-dimorphic (e.g. aphids: Braendle et al., 2006, crickets: Zera and Denno, 1997) and wing-monomorphic insects (e.g. beetles: Dmitriew et al., 2009, butterflies: Pellegroms et al., 2009). Such change not only effect male foraging activity but also increases male mating success in scramble competition due to his greater agility.

Therefore, selection might favour increased investment in flight and flight related traits in poor conditions.

The effects of nutrient limitation on reproductive traits appear contradictory. Sexual selection typically favours enlargement of genitalic and secondary sexual traits, such as weapons used in intrasexual competition (e.g. beetles: Johns et al., 2014) or a signal ornaments to attract females (e.g. damselflies: Outomuro et al., 2014; Mediterranean fruit fly: Rodriguero et al., 2002). However, when resources are limited, emphasis of one trait may result in constraint of another. Given that the traits used in intrasexual competition and intersexual choice are different (e.g. Candolin, 2005) males have an opportunity to increase their reproductive success using alternate reproductive tactics that may sometimes be expressed in response to environmental conditions. For example, in the Indian mealmoth, *Plodia interpunctella*, under *ad libitum*, males that encounter few females develop large heads and thoraxes that aid in mate finding, whereas higher female densities favour more investment in gamete production (Gage, 1995). Such change in investments also led to correlated changes in other life-history traits over generations due to selection (e.g. Yamane et al., 2010).

In general, genitalia show a weaker response to environmental variation than other non-genitalic traits (e.g. Cayetano et al., 2011; House and Simmons, 2007; Simmons et al., 2009). Male genitalic structures are complex and have specialised functions in mating behaviour (Eberhard, 1985). For example, in Lepidoptera, external genitalia (valva and uncus) are important in the coupling process and internal genitalia (aedeagus and vesica) lock sexes together to facilitate sperm transfer (Mikkola, 2008). During copulation, external and internal genitalia interlock with female genital parts and genitalia of either sex can independently or in combination influence male mating and fertilization success (see Simmons, 2014 for references).

Other primary sexual structures such as testis and accessory glands produce sperm and ejaculate components. Sperm production is limited, costly and its replenishment takes time (Dewsbury, 1982; Trivers, 1972). When a male cannot produce a full ejaculate it refrains from mating (e.g. Bissoondath and Wiklund, 1996; Reinhardt et al., 2011), which has a dramatic effect on his mating frequency (the measure of male reproductive potential). Several studies have shown that larval rearing conditions affect the sperm number, ejaculate size and mating frequency in a range of insects (e.g. Indian mealmoth *P. interpunctella*, Gage and Cook, 1994; army worm *Pseudaletia separata*, He and Miyata, 1997; Indian mealmoth *P. interpunctella*, Lewis et al., 2011; almond moth *Cadra cautella*, McNamara et al., 2010) that may trigger a trade-off between different traits. Therefore, understanding how the juvenile condition affects the adult phenotype and reproductive investment together is important.

The varied reproductive strategies used by to gain access to females provides an opportunity to test predictions on how resource-limited males alter their allocation pattern in morphological (ecological and sexual) traits and develop a mating strategy that increases reproductive success. I tested the prediction that resource-limited males would not alter their investment in genitalia but would increase investment in ecological traits (wings, head and thorax) useful for foraging and mate-searching and decrease investment in sperm number.

Alternatively, males may invest more in reproductive traits (sperm number in each mating) but decrease mating frequency.

Materials and methods

Study species

The Mediterranean flour moth, *Ephestia kuehniella* is a stored product pest (Hill 2002). The larval developmental period lasts for 20–30 days (chapter 2), with nutrients needed for adult reproduction being obtained during that stage as adults do not feed (Norris and Richards, 1932). Both sexes sexually mature soon after emergence (Calvert and Corbert, 1973) and prefer to mate with large partners (Xu and Wang, 2009a). Males can mate on an average up to 8 times in their lifetime and live for up to 14 days on an average. In each mating, males transfer a single spermatophore consisting of sperm and accessory gland products.

Laboratory stock cultures were maintained in plastic containers (8 cm in diameter and 10 cm high). Larvae were reared on a diet of wholemeal wheat flour (43.5%), maize meal (43.5%), brewer's yeast (3%) and glycerine (10%) (Lima-Filho et al., 2001), at a density of 200 larvae to 50 g of food (chapter 2) and kept at 25 ± 1°C and 60 ± 10% relative humidity on a 14:10 h (light:dark) cycle. Eggs less than 24 h old collected from stock culture females were used to establish experimental groups at four population densities: 100, 200, 400 and 800. The randomly selected eggs were placed in clean containers with 50 g of the dietary medium for all densities. The amount of diet was kept constant for all densities, creating a range of food availability conditions. Mature pupae were collected and placed in individual glass vials (2 cm in diameter and 7.5 cm high). After emergence, adults were sexed.

Thirty adult males from each density were randomly selected, anesthetized with CO_2, weighed individually using an electronic dual range balance (Mettler AE100, Mettler-Toledo Inc., Switzerland) with a precision of 0.1 mg and then frozen at —20°C. After 24 h, forewing length (from the point of articulation to the distal end of the forewing), head and thorax width were measured for each individual (Figure 1). They were then dissected so that valva length and aedeagus length (from the point of sclerotization to tip) could be measured. All morphological traits were measured using images captured by a USB 2.0 Video/Audio Grabber (Lindy Electronics Ltd, UK) with Universal Desktop Ruler software (AVPSoft, USA).

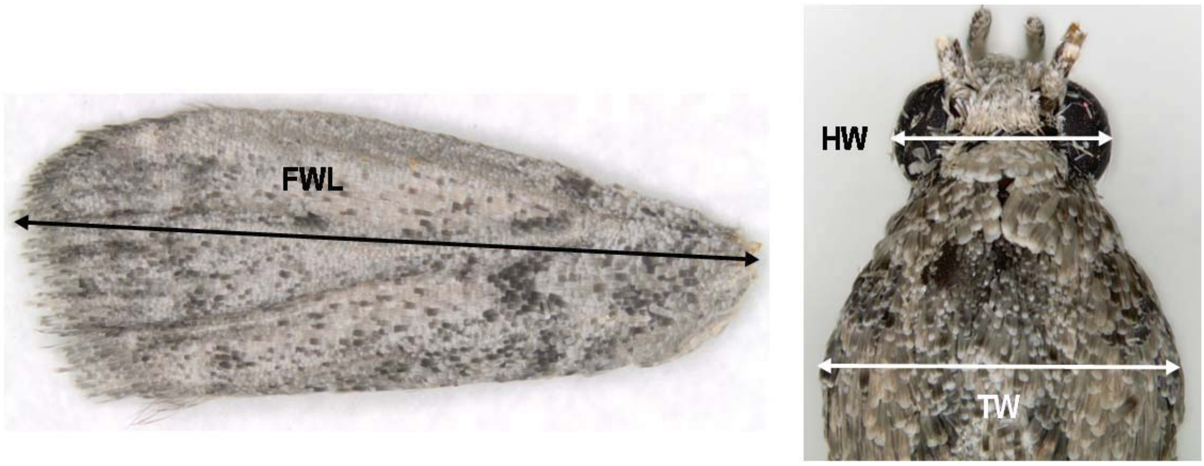

Figure 1. Morphological measures made on male *Ephestia kuehniella*. FWL, HW and TW represent forewing length, head and thorax widths, respectively.

Male mating success

To determine the lifetime mating success of males in different conditions, 13 one day old virgin males were randomly selected from each of the 200, 400 & 800 population density cultures. Prior observations showed that individuals that developed at population densities of 100 and 200 larvae had similar levels of fitness (Chapters 2 & 3). Hence the population density of 100 larvae was excluded in the present experiment. At the start of the scotophase, each male was provided with a one day old virgin female taken from the same experimental population density. If copulation occurred, the female was removed after mating termination, killed and dissected for spermatophore extraction. The number of apyrene (non-fertile) and eupyrene (fertile) sperm in the spermatophore was counted as described below. A male *E. kuehniella* requires 24 h recovery time to produce a full spermatophore. Therefore, after 24 h each male was provided with another one day old virgin female and the same procedure was repeated for every 24 h until the male died. For each male, mating frequency and adult longevity were recorded.

Sperm count

To count the number of eupyrene and apyrene sperm transferred to a female during mating, females were frozen at –20 °C immediately after mating and dissected for spermatophore extraction. On a microscope slide the spermatophore was ruptured with a fine needle to release the sperm. During copulation, eupyrene sperm were transferred in bundles and each bundle consisted of 256 individual spermatozoa. Therefore, to obtain the total number of eupyrene sperm transferred, the number of bundles was counted under x40 magnification and then multiplied by 256. After that the sperm mass was washed with Barth saline into a specimen tube. The volume is made up to 30ml by diluting with distilled water. Six separate 10μl subsamples were taken from the sample using a Gilson autopipette and left to dry on a microscope slide. The apyrene sperm in each dried subsample was counted using dark field phase-contrast microscopy at x100 magnification. The average of the counts made from each 10μl subsample was then multiplied by the dilution factor to obtain the total number of apyrene sperm transferred (Cook and Wedell, 1996).

Statistics

All statistical analyses were conducted using SAS version 9.3. Data on lengths and widths of different morphological traits, male mating frequency, adult longevity and numbers of eupyrene and apyrene sperm were analysed using ANOVA followed by Tukey's HSD test for multiple comparisons. Regression analysis was performed to determine the relationship between the size of various morphological traits and body mass. The slopes of the regression lines were compared using ANCOVA. The Shapiro-Wilk test was used to test the normality of the data.

Results

Adult males reared at densities of 100 and 200 larvae were significantly heavier than those reared at population densities of 400 and 800 individuals ($F_{3,113}$ = 20.04, $P < 0.0001$; Table 1). There was a significant effect of population density on forewing length ($F_{3,113}$ = 3.03, P = 0.033), head width ($F_{3,113}$ = 7.54, P = 0.0001) and thorax width ($F_{3,113}$ = 28.48, P <0.0001) with the smallest sizes recorded at the highest (800) population density (Table 1). However, the average valva and aedeagus lengths did not differ significantly among males reared at different densities (valva: $F_{3,102}$ = 1.26, P = 0.291; aedeagus: $F_{3,103}$ = 0.28, P = 0.838) (Table 1).

Table 1. Mean (±SE) body mass (mg) and morphological trait sizes (mm) of *Ephestia kuehniella* males reared at four population densities. In each row, means followed by the same letter were not significantly different at *P* = 0.05.

Trait	100	200	400	800
Body mass	15.21 ± 0.34 a	15.63 ± 0.34 a	13.89 ± 0.28 b	12.31 ± 0.37 c
Forewing length	8.76 ± 0.08 a	8.64 ± 0.08 a	8.52 ± 0.17 ab	8.30 ± 0.09 b
Head width	1.25 ± 0.01 a	1.25 ± 0.01 a	1.22 ± 0.01 a	1.19 ± 0.01 b
Thorax width	2.19 ± 0.03 a	2.20 ± 0.03 a	2.15 ± 0.03 b	2.13 ± 0.02 b
Valva length	1.34 ± 0.02 a	1.32 ± 0.01 a	1.33 ± 0.03 a	1.29 ± 0.02 a
Aedeagus length	1.55 ± 0.03 a	1.53 ± 0.03 a	1.56 ± 0.03 a	1.53 ± 0.02 a

With increasing body mass, forewing length increased significantly at population densities of 400 and 800, but body mass had no significant effect on forewing length at population densities of 100 and 200 (Table 2). ANCOVA further indicated that forewing length increased significantly faster at higher population densities of 400 and 800 than at lower population densities of 100 and 200 ($F_{3,109}$ = 2.72, P = 0.048) (Figure 2a). The size of the other morphological traits did not significantly increase with body mass ($P > 0.05$), except thorax width at population density of 800 and head width at higher population densities of 400 and 800 ($P < 0.05$) (Table 2). The slopes of linear regressions of these morphological traits over body mass did not differ significantly between population densities (for thorax: $F_{3,109}$ = 0.41, P = 0.749; head: $F_{3,109}$ = 1.10, P = 0.351; valva: $F_{3,98}$ = 0.19, P = 0.905; aedeagus: $F_{3,98}$ = 0.70, P = 0.553) (Figure 2 b-e).

Table 2. The relationship between log body mass (x, mg) and log morphological trait size (y, mm) in width and length) of *Ephestia kuehniella* males that emerged from different population densities. Significant terms are indicated in bold. Regression lines were further compared to determine their slope difference (Figure 2).

Morphological trait	Population density	Linear regression	R^2	*F*	*P*
Forewing length	100	y = 0.86 + 0.07 x	0.026	1,24 = 0.69	0.415
	200	y = 0.80 + 0.12 x	0.089	1,27 = 2.73	0.110
	400	y = 0.47 + 0.40 x	0.189	1,24 = 6.53	**0.016**
	800	y = 0.57 + 0.31 x	0.768	1,25 = 89.53	**<0.0001**
Head width	100	y = 0.02 + 0.06 x	0.024	1,24 = 0.64	0.432
	200	y = —0.04 + 0.11 x	0.019	1,27 = 2.99	0.095
	400	y = —0.19 + 0.24 x	0.241	1,24 = 8.88	**0.006**
	800	y = —0.12 + 0.18 x	0.318	1,25 = 12.60	**0.001**
Thorax width	100	y = 0.14 + 0.17 x	0.073	1,24 = 2.03	0.165
	200	y = 0.19 + 0.13 x	0.063	1,27 = 1.87	0.182
	400	y = 0.28 + 0.05 x	0.005	1,24 = 0.14	0.708
	800	y = —0.07 + 0.20 x	0.234	1,25 = 8.25	**0.008**
Valva length	100	y = —0.06 + 0.15 x	0.047	1,24 = 1.17	0.289
	200	y = 0.00 + 0.10 x	0.051	1,27 = 1.44	0.241
	400	y = —0.12 + 0.21 x	0.142	1,23 = 3.79	0.064
	800	y = —0.01 + 0.11 x	0.033	1,24 = 0.82	0.374
Aedeagus length	100	y = 0.26 + 0.00 x	0.000	1,24 = 0.00	0.997
	200	y = 0.18 + 0.01 x	0.000	1,27 = 0.00	0.971
	400	y = 0.01 + 0.15 x	0.070	1,24 = 1.74	0.201
	800	y = 0.16 + 0.02 x	0.002	1,25 = 0.06	0.815

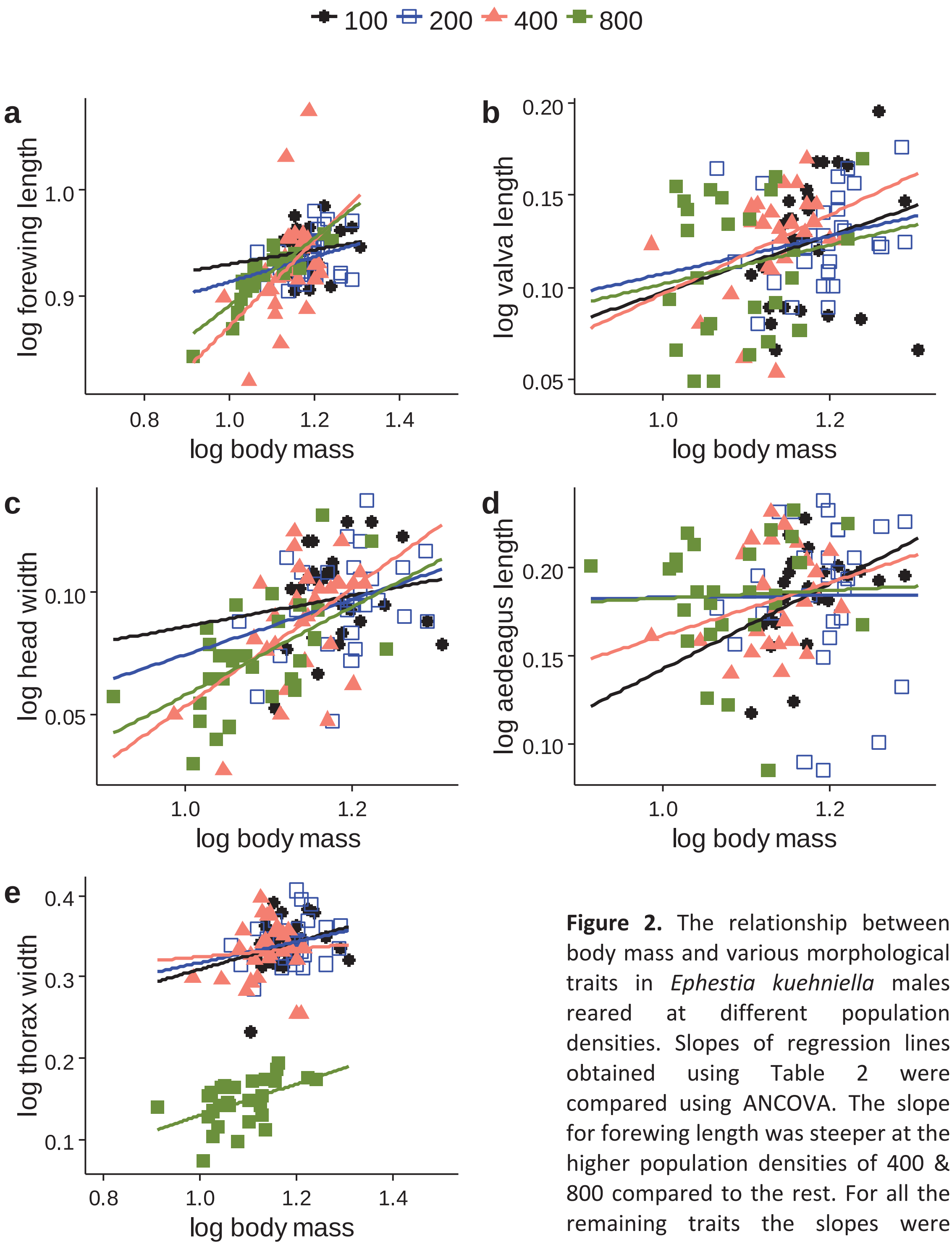

Figure 2. The relationship between body mass and various morphological traits in *Ephestia kuehniella* males reared at different population densities. Slopes of regression lines obtained using Table 2 were compared using ANCOVA. The slope for forewing length was steeper at the higher population densities of 400 & 800 compared to the rest. For all the remaining traits the slopes were homogeneous. Adult body mass was measured in mg and the morphological traits in mm.

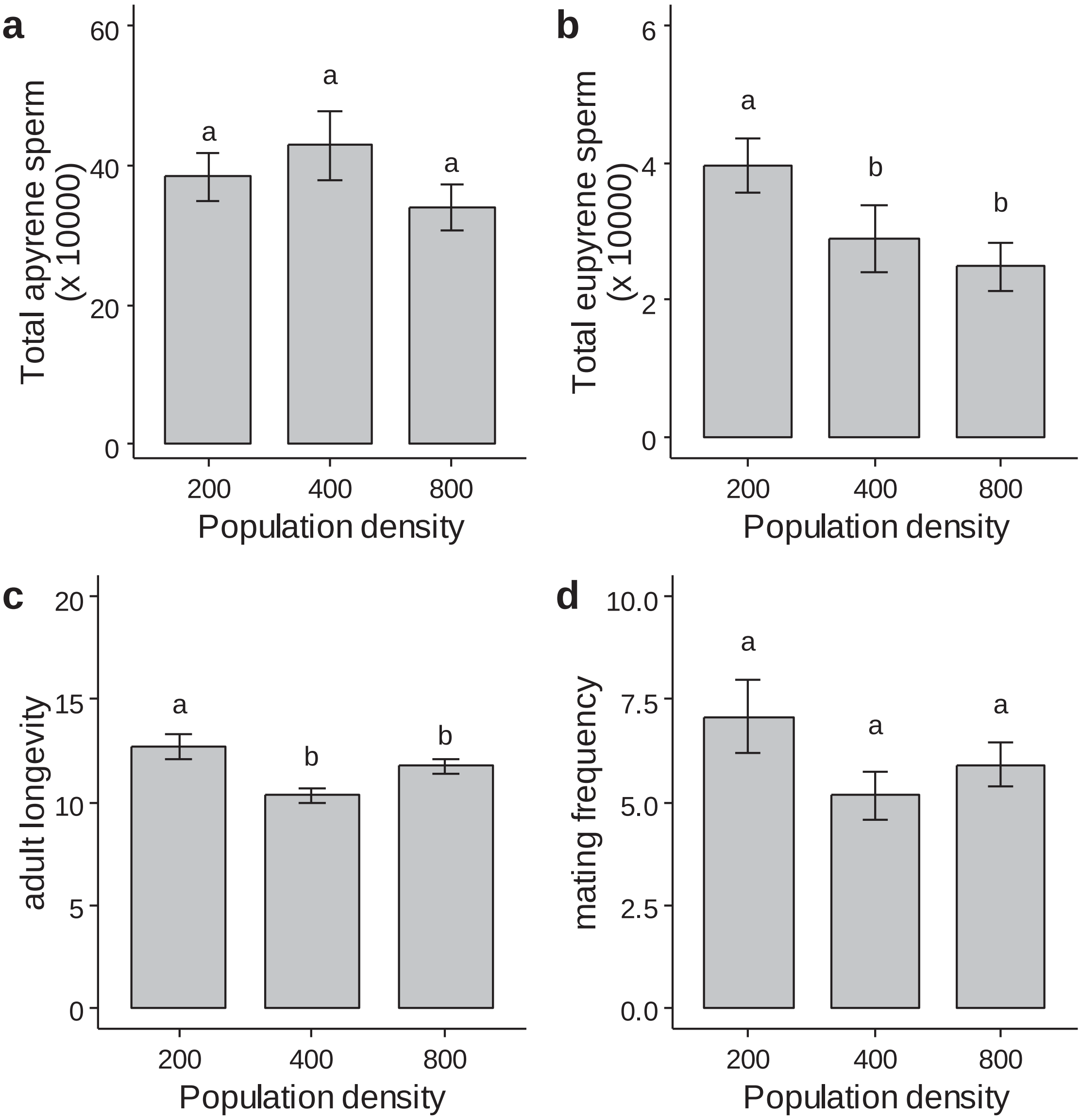

Figure 3. Effect of population density on *Ephestia kuehniella* male reproductive success: total numbers of apyrene (a) and eupyrene (b) sperms produced, mating frequency (d) and adult longevity (c). Error bars represent standard errors about the mean. For each parameter, bars with different letters are significantly different ($P < 0.05$).

There was no significant difference in the average numbers of apyrene sperm produced by males reared at different densities ($F_{2,32} = 1.29$, $P = 0.29$) (Figure 3a) but males reared at a population density of 200 produced more eupyrene sperm ($F_{2,32} = 3.33$, $P = 0.04$) (Figure 3b) and lived for significantly longer periods than those at population densities of 400 & 800 ($F_{2,32} = 7.53$, $P = 0.002$) (Figure 3c). Regardless of the number of eupyrene sperm produced and adult longevity, males taken from different densities mated a similar number of times during their lifetime ($F_{2,32} = 1.98$, $P = 0.16$) (Figure 3d).

Discussion

Population density during early development stages can have a profound impact on adult phenotype (Atterholt and Solensky, 2010; Bauerfeind and Fischer, 2005; Vishalakshi and Singh, 2008). These effects are most probably induced by a shortage of food at higher densities (e.g. Hooper et al., 2003), which is exerted by growing larvae competing for food and space (e.g. Hoffmann and Loeschcke, 2006) reducing the time available for feeding (e.g. Tsuda and Yoshida, 1985). As a result, the amount of available resources needed for development of traits is disproportionately reduced. With limited resource pool the allocation patterns to different traits differs leading to changes in the allometric relationship between body mass and different morphological traits (e.g. Dmitriew et al., 2009; Tigreros et al., 2013).

Consistent with these studies, I found that adult moths emerged from the population density (800) were lighter in weight and had small forewings, heads and thoraxes than those reared at the lower population densities (100 and 200) (Table 1). Population density had no effect on the allometric relationship between body mass and the widths of thorax and head (Figure 2c & e). However, the slopes of male forewing length differed among densities and males at higher population densities (400 and 800) had larger wings relative to their body mass (Figure 2a). These results are in contrast with the female response to increasing population density, which did not involve a change in wing morphology (see chapter 3). The lengths of the valva and aedeagus of male *E. kuehniella* that emerged from different population densities did not differ significantly (Table 1) and there was no significant difference in the relationship between body mass and other traits (Table 2): slopes of these trait relationships being homogenous (Figure 2b & d).

In butterflies, it has been shown that food-deprived adults tend to alter their body mass/wing shape in a way that alters wing area and loading (Berwaerts et al., 2002; Pellegroms et al., 2009; Thomas et al., 1998). Similarly, in *Drosophila* when larvae are stressed through larval crowding, adults developed long and pointed wings (Bitner-Mathe and Klaczko, 1999). Alteration in wing morphology typically represents a difference in dispersal potential (Angelo and Slansky, 1984). In the present study, as predicted, resource allocation shifted in response to food stress (Figure 2a). Wing loading decreased with the increase in wing length/body mass ratio (Dudley, 1999). This may affect flight efficiency and thus duration. Although flight is energetically expensive, lower wing loading enables even individuals in poor condition to fly with reduced flight costs. This indicates an adaptive strategy expressed in experimentally food stressed *E. kuehniella* males to move away from low quality habitats and find new places that may increase their fitness.

Another possible explanation could be that under bad conditions a population becomes male-biased due to greater mortality, migration, or non-availability of mates during oviposition. Under such conditions, male reproductive success increases with his mate-searching ability. It can be presumed that larger wings developed by males emerged from higher population densities of 400 & 800, may increase the probability of mate finding due to greater agility. This would be an adaptive strategy for successful mate location to mitigate the disadvantages of being small when developed in a dense populations as has been influenced by food-deprived butterfly *Pararge aegeria* (Berwaerts et al., 2002; Pellegroms et al., 2009) and moths that experienced different population structures (e.g. Indian mealmoth *P. interpunctella*, Gage, 1995). Alternative reproductive tactics such as sneaky behaviour and greater agility developed by small males have evolutionary importance (see Stockley et al., 1994). Whether males emerged from high and low densities employ different mating tactics is worth examining.

In semelparous species energy reserves are fixed and resource-limited males at higher population densities of 400 & 800 are even more resource constrained, indicating a potentially driving trade-off in resource allocation. In the present study, the number of eupyrene sperm produced by males emerged from higher population densities (400 & 800) was on average lower compared to males at population density of 200 (Figure 3b) but there were no significant differences in the apyrene sperm production (Figure 3a). This was consistent with studies on Indian mealmoth, *P. interpunctella* showing that nutrient-limited males produced fewer eupyrene sperm (Gage and Cook, 1994). Sperm production is costly (Dewsbury, 1982) but males of many insects transfer more sperm to females in each mating than is needed to fertilize available eggs (Arnqvist and Nilsson, 2000). In *E. kuehniella*, a decrease in eupyrene sperm number did not have an effect on female fertility. Therefore, in resource-limited *E. kuehniella* males trade-off might have occurred between investment in eupyrene sperm and mating frequency and longevity.

At higher densities, male reproductive success is dependent on migration and mate-searching ability (e.g. Pomfret and Knell, 2006) and males investing more in survival can provide a net benefit as adults that live long have more chances of finding a mate. In *E. kuehniella* I demonstrate that males at higher population density (400 & 800) lived for 12–13 days slightly shorter on average than males at population density of 200, which lived for 14 days (Figure 3c), however, males mated a similar number of times (Figure 3d). The alteration in wing morphology may aid food-restricted males in mate-searching.

The pattern in male *E. kuehniella* contrasts with *P. interpunctella* in terms of apyrene sperm production. This can be explained by different experimental conditions; male *P. interpunctella* experienced food shortage but not larval crowding compared to *E. kuehniella* which were food-stressed and crowded. At higher densities, polygamy is common (Gage, 1995). As a result males experience greater sperm competition risk, which favours production of large numbers of apyrene sperm. In Lepidoptera, apyrene sperm production incurs less cost and acts as cheap filler that reduces female receptivity (Silberglied et al., 1984). Production of large number of apyrene sperm is therefore a male adaptation to increase the female remating interval. These findings suggest that an adult male respond to the juvenile rearing environment and adds evidence to the growing literature showing males alter their spermatogenesis and sperm allocation strategies in response to juvenile environment (He and Miyata, 1997; Lewis et al., 2011; McNamara et al., 2010).

I found that the allometries of the valva and aedeagus did not differ (Figure 2b & d), and this is consistent with inferences that genitalia are insensitive to environment and body mass (Tadler, 1999) due to canalisation (Eberhard et al., 1998). In treehoppers *Enchenopa binotata* (Rodriguez and Al-Wathiqui, 2011), water striders *Aquarius remigis* (Bertin and Fairbairn, 2007) and the dung beetle

Onthophagus taurus (Simmons et al., 2009) environmental conditions had the weakest effect on genital traits out of a suit of characters studied. This indicates that intermediate sizes are favoured through stabilizing selection (see Hosken and Stockley, 2004).

In *E. kuehniella*, male valva have an important function during early coupling: to grasp the female abdomen. The sclerotized structures, valva and tegumen interact with the corresponding structures of the female to secure coupling allowing aedeagus insertion (Drummond, 1984; Norris and Richards, 1932). An increase in the length of male valva demands a co-evolutionary increase in the size of female genital and other male genital parts involved. On the other hand small valva cannot reach the female genital openings and may result in failed coupling and reduce male intromission success. In the white-tailed zygaenid moth *Elcysma westwoodii*, clasper asymmetry decreased the chances of grasping the female abdomen (Koshio et al., 2007). In true bug *Stenomacra marginella*, Moreno-Garcia and Cordero (2008) experimentally manipulated the valva size by partial and complete amputation and found that this manipulation resulted in a decrease of intromission success by more than half. It is also possible that in *E. kuehniella* secure coupling may reduce the probability of mating take-overs by rival males. Therefore, it is most likely that males with appropriate valva length have an advantage and may be successful in intra and inter-sexual competition.

Aedeagus morphology evolves mainly due to intersexual selection and thus can determine mating and fertilization success (see Simmons, 2014). In *E. kuehniella*, after coupling the aedeagus is inserted through the ostium bursae into the corpus burase. The male cornuti locks with sclerotized plates in the cervix bursae of the female to keep the aedeagus in position (Norris and Richards, 1932). At this stage, mating is sometimes terminated by females. This probably happens if the aedeagus is too small and fails to reach the female bursa copulatrix and therefore cannot initiate the copulation. In the pairs that manage to establish successful mating interaction, females assess the aedeagus size and continue to mate with males that provide the right fit and stimulation.

In the ground beetle *Carabus insulicola*, aedeagus length affects insemination success via spermatophore size, place of deposition and copulation duration (Takami, 2003; Takami and Sota, 2007). Likewise, in the blow fly *Phormia regina*, Stoffolano et al. (2000) found that small males transferred fewer sperm to large females and attributed this strategy to the effect of the small aedeagi. In *E. kuehniella*, once mating is initiated, spermatophore formation and sperm transfer occur inside the aedeagus which is then placed in the female bursa copulatrix (Drummond, 1984; Norris and Richards, 1932). The deposition of spermatophore in the right position is important as it facilitates the spermatophore opening and movement of sperm to the storage and fertilization sites. Males with a large aedeagus place the spermatophore deep into the bursa copulatrix where the corresponding female structures to open the spermatophore are absent. This would suggest that an intermediate size is advantageous in post-copulatory sexual selection as that increases the fertilization success.

In conclusion, this study demonstrates that adult body mass, sizes of wing, head and thorax but not lengths of valva and aedeagus of male *E. kuehniella* are affected by larval growth conditions. At metamorphosis individuals have the opportunity to adjust their resource allocation pattern. Larvae reared at higher population densities (400 & 800) emerged with longer wings than those reared at lower population densities of 100 & 200. Changes in wing morphology represent dispersal potential. Eupyrene sperm production decreased with increasing population density, however, the numbers of apyrene sperm and matings did not differ between the densities. There were no differences in the allometries of valva and aedeagus. Together these data suggests that males maintain their investment in traits that have a drastic effect on intromission success at the expense of gamete production.

CHAPTER 5

Are mates from more demanding environments preferable?

Abstract

In general, individuals that develop in good environments are in good condition and those developed in poor environments (food shortage) are in poor condition due to reduced resource availability. However, due to variation in resource acquisition ability among individuals, condition differs and individuals developed in food stressed environment may still achieve similar body mass as some individuals developed at *ad libitum* conditions. Previous evidences shows that good-conditioned individuals were readily accepted as mating partners however, when controlled for individual condition across multiple environments, are males and females from poor environment accepted as mating partners in mate selection process? To test this prediction here, I reared Mediterranean flour moth, *Ephestia kuehniella* under low population density (200; food was *ad libitum)* and high population density (800; food was limited). As body mass and adult reproductive success are positively correlated, body mass was used to select individuals for experiments. Individuals from high population density (800) and low population density (200) that achieved similar body mass were randomly selected and paired. Males from low population density (200) courted females for longer compared to males from high population density (800), because females readily accepted the latter males as mating partners. Similarly in male mate choice, despite females from low population density (200) calling first, males preferred to mate with females from high population density (800). In the subsequent series of experiments, it was found that females from high population density (800) had greater fecundity and fertility than mass-matched females from low population density (200) while male reproductive output, (i.e., sperm numbers and mating

frequency) did not differ between mass-matched males from 200 and 800 population densities. These results suggest that both males and females discriminate their partners and by mating with genotypes that are subjected to selection by environmental stress both sexes obtain direct and possibly indirect genetic benefits to their offspring as well.

Introduction

Poor nutrition early in life has negative effects on adult body mass, lifespan, secondary trait expression and reproduction in many invertebrates including spiders (Kleinteich et al., 2015), butterflies (Bauerfeind and Fischer, 2005), beetles (Fouad et al., 2000), grasshoppers (Wall and Begon, 1986), flies (Macia, 2009), etc. It has also been shown that condition-dependent expression of sexual traits (e.g. David et al., 2000; Tomkins et al., 2004) and changes in allometric relationships among traits in response to environmental variation can influence reproductive success (e.g. Boggs and Freeman, 2005; Pellegroms et al., 2009; Saastamoinen et al., 2010) and enhance survival (Emlen, 1996; Frankino et al., 2005).

It has been assumed that low quality environments reduce individual condition and fitness, but in the stalk-eyed fly *Cyrtodiopsis dalmanni*, it was demonstrated that genetic quality can mask environmental effects so that in any environment individuals of high genetic quality have greatest fitness (David et al., 2000). High quality individuals achieve greater resource acquisition and assimilation (e.g. David et al., 2000), resist pathogens (e.g. Westneat and Birkhead, 1998), manage oxidative stress (e.g. von Schantz et al., 1999) or have better survival (e.g. Win et al., 2013), resulting enhanced individual fitness. Due to genotypic differences in resource acquisition and allocation, individuals developed in stressful environment achieve similar condition as individuals at normal environment (e.g. Wall and Begon, 1987). Light weight individuals at low density are often sexually less receptive (e.g. female black field cricket *Teleogryllus commodus* Hunt et al., 2005) and take longer to mate (e.g. male *Bicyclus anynana* butterflies; Lewis et al., 2010). These individuals are actively rejected as mating partners due to their underlying genetic quality not phenotype (Fisher, 1915; Kirkpatrick, 1996). Variation in condition results in variation in fitness, because offspring is limited by condition. Theory suggests that males and females prefer to mate with individuals in good condition (Andersson, 1994). This indicates that individuals choosing their reproductive partners based on condition are selecting for genes that are responsible for efficient resource acquisition and allocation regardless of the environment they experienced.

Mating preferences are usually expected to evolve to recognise the genetic quality of partners (Andersson, 1994). It has been suggested that males are the less choosy sex because they produce the cheaper gametes, but males exert choice when there is a greater variation in female reproductive quality, male mating investment and operational sex ratio (Edward and Chapman, 2011). This is because sperm production, while cheaper per gamete is nevertheless limited and it takes time to replenish sperm and produce a complete ejaculate (Dewsbury, 1982; Trivers, 1972). When selecting mates, males typically use traits that indicate female fecundity or viability (Bonduriansky, 2001).

In the present study, I assessed the influence of larval stress on mate choice decisions in the Mediterranean flour moth, *Ephestia kuehniella* and postulated that females and males distinguish potential mates based on the mates' juvenile environment. The differences in eupyrene and apyrene sperm numbers and mating frequency between weight-matched males, and fecundity of weight-matched females were also compared. Determining these provides information about the direct benefits that males and females obtain by mating with prospective mates.

The Mediterranean flour moth, *E. kuehniella* is a Lepidopteran pest of stored products. Larvae feed on flour and animal products (Rees and Rangsi, 2004). Food shortage during the juvenile stage has a negative effect on developmental period, pupal mass, survival and female fecundity (chapter 2). Mating occurs during night, when females assume a calling posture by protruding their ovipositor and releasing a sex pheromone. Males approach the calling female while fanning their wings and initiate courtship (Calvert and Corbert, 1973). It would therefore be expected that males who reach a female more quickly have the highest chances of mating. Abdominal thickness is an indicator of female lifetime fecundity, which is approximately 300 eggs (Xu and Wang, 2010), and food stressed females have larger abdomens relative to body mass compared to those at normal conditions (chapter 3). Therefore, it was predicted that females from high population density would be preferred by males.

Materials and methods

Laboratory stock cultures of the Mediterranean flour moth were maintained in plastic containers (8 cm in diameter × 10 cm in high). In each container, 200 larvae were reared on 50 g of diet comprising wholemeal wheat flour (43.5%), maize meal (43.5%), brewer's yeast (3%) and glycerine (10%) (Lima-Filho et al., 2001) at 25 ± 1°C and 60 ± 10% relative humidity with a photoperiod of 14:10 h (light:dark) cycle. Eggs less than 24 h old were harvested from stock cultured mated females and used to establish three experimental population densities: 100, 200 and 800. These were provided with 50 g of diet and maintained in standard conditions. Mature pupae were collected, weighed using an electronic dual range balance (Mettler AE100, Mettler-Toledo Inc., Switzerland) with a precision of 0.1 mg and placed individually in separate glass vials (2 cm diameter x 7.5 cm high). Adults that emerged from the pupae were sexed and used in the following experiments. Two scenarios were examined. One in which individual males had a choice of female mates and the second in which individual females had a choice of mates. In both cases adults that were to be given a choice of mates were derived from the 100 population density. Pupal mass of these males and females were on average 23.02 ± 2.65 and 25.29 ± 2.40 mg, respectively. Prospective mates were taken from 200 and 800 population densities and weight-matched. All the adults used were one day old virgins. Mate choice trials were conducted during the scotophase under 30W incandescent red light bulb that facilitated observation. Prior to introduction, prospective mates were marked with different colours (white or orange, Magruder Colour Company, USA) for identification.

Each male was offered two females taken from different population densities (200 & 800) that did not differ significantly in weights ($P > 0.05$). In each replicate mating activities were recorded: first female to call, male courtship frequency (total number of times (successful + unsuccessful attempts) a male has courted a female during mate choice process) towards each female and mate choice decision. Thirty one replicates were performed. In addition, I tested for fecundity and fertility differences between the weight-matched females from different population densities. In separate containers, 20 females were paired with 20 males for their lifetime. From each female, eggs were collected daily into Petri dishes until she died. The total number of eggs (fecundity) and eggs with black dots (fertility) were counted.

Each female was given two weight-matched males from different density populations (200 & 800). The mating activities recorded were which male fanned first, courtship frequency (total number of times (successful + unsuccessful attempts) and courtship duration (the amount of time in seconds that male courted the female in each courtship was added up to calculate the courtship duration), and the male that achieved mating. Thirty three replicates were performed.

To document differences in lifetime mating success of weight-matched males from 200 and 800 larval populations, 20 males were caged separately in individual glass vials. At the start of the scotophase, each male was provided with one day old, average sized, virgin female randomly taken from a 100 density population. The procedure was repeated each day until the male died. After copulation ended, females were dissected for spermatophore extraction. The numbers of eupyrene and apyrene sperm were counted (Cook and Wedell, 1996). For each male, mating frequency was also recorded.

Statistics

The impact of population density on male and female mate choice was determined using logistic regression analysis. Chi-square tests were used to analyse the effect of population density on male courtship and female calling. Differences between the pairs of potential mates in terms of courtship frequency and duration, eupyrene and apyrene sperm, mating frequency, fecundity and fertility were compared using paired *t* tests. All analyses were performed in SAS version 9.3.

Results

Male mate choice

Females from the lower population density (200) started calling first (χ^2 = 8.38, P = 0.004). Males courted females from both population densities a similar number of times (χ^2 = 3.29, P = 0.069), but significantly preferred to mate with 800 females over 200 females (χ^2 = 5.23; P = 0.022).

Although there was no significant difference in the weight of females from the two population densities, females developed in the denser larval population laid significantly more eggs and more fertile eggs than those developed in lower density conditions (Fecundity: t = —2.36, P = 0.042; Fertility: t = —2.36, P = 0.043) (Table 1).

Table 1. Comparison of lifetime reproductive output (fecundity (total eggs laid) and fertility (total fertile eggs laid)) of mass-matched female *Ephestia kuehniella,* taken from low (200) and high (800) density.

Density	Fecundity	Fertility
200	196 ± 16.40	195.6 ± 16.38
800	245 ± 15.78	243.3 ± 15.71

Female mate choice

Male courtship activity had a significant effect on mate choices of females (χ^2 = 8.79, P = 0.003). There was no significant bias in which male initiated courtship first (χ^2 = 0.73, P = 0.394), however, females preferred to mate with males from the high density population (800) (χ^2 = 4.14, P = 0.042). Pair-wise comparisons shows that the frequency of courtship displayed by the two males did not differ statistically (t = 1.41, P = 0.195) (Table 2), but males from the low density population courted for longer than males from high density population (t = 3.68, P = 0.006) (Table 2). There was no significant difference in the number of lifetime matings achieved by males from low and high density (t = —1.35, P = 0.235) (Table 2) and males transferred similar numbers of eupyrene (t = —0.82, P = 0.447) and apyrene (t = —0.38, P = 0.721) sperm (Table 2).

Table 2. Comparison of male behavioural activities (courtship frequency and courtship duration recorded during mate choice) and lifetime reproductive success (eupyrene and apyrene sperm produced and mating frequency) of mass-matched male *Ephestia kuehniella,* taken from low (200) and high (800) density.

Density	Courtship frequency	Courtship duration	Mating frequency	Total eupyrene sperm	Total apyrene sperm
200	2.33 ± 0.37	50.44 ± 11.96	2.00 ± 0.58	17152 ± 5407.19	251583 ± 78087.7
800	1.67 ± 0.24	24.56 ± 6.85	2.67 ± 0.49	21504 ± 4166.85	286000 ± 65126.2

Courtship frequency includes successful + unsuccessful courts made by a male. Courtship duration represents the total time period (seconds) spent in courtship activity.

Discussion

Theory predicts that males and females prefer to mate with partners of high quality (Andersson, 1994) to obtain direct and indirect benefits. In the present study, it was found that males from low (200) and high (800) population densities juvenile environment initiated courtship at the same time, however, *E. kuehniella* females preferred to mate with individuals that developed at high population density (800) over individuals from low population density (200), when their weights were matched. Observations in male mate choice in *E. kuehniella* also showed a bias. Although females from low population density (200) started to call first, they were mostly unsuccessful in obtaining a mating and males significantly choose females at high population density (800) as mating partners. This study suggests that matings in *E. kuehniella* were non-random and that mate choice outcome was influenced by the rearing conditions experienced by the potential mates during their juvenile stage. Furthermore, it was found that males developed at high population density (800) produced similar number of eupyrene and apyrene sperm and had similar mating frequency as males from low population density (200) (Table 2) whereas females developed at high population density (800) had greater reproductive output (Table 1). This indicates good resource acquisition, assimilation and allocation ability of individuals developed at dense larval population.

Previous research showed that in female Lepidoptera there is a positive correlation between resource acquisition and body size and in turn abdomen size (Kivela et al., 2012; Wickman and Karlsson, 1989). Females allocate most resources to the abdomen, an indicator of female fecundity (Wickman and Karlsson, 1989). Males intensively court females that carry more eggs (the Sulphur butterfly *Eurema hecabe*, Takanashi et al., 1997) and readily accept more fecund females as mating partners as in Lepidoptera and other species such as preying mantid *Pseudomantis albofimbriata* (Barry, 2010) and *Drosophila melanogaster* (Byrne and Rice, 2006). Consistent with these studies, male *E. kuehniella* choose to mate with females at high population density (800) that are more fecund. By selecting for more fecund females, *E. kuehniella* males are more likely to maximise their reproductive success through direct fecundity benefits and indirect good genes as body size is a heritable trait in this species (chapter 6).

Males can assess female reproductive quality using pheromone cues. For example, in preying mantid, males discriminate females on pheromone quality and select females with greater reproductive potential (Barry, 2010). It is possible that male

E. kuehniella obtained chemical cues via pheromones that indicate female quality. In *E. kuehniella* females release pheromones prior to mating and males follow pheromone traces to locate and court the female and then mate successfully (Phelan and Baker, 1990). Pheromone production and egg maturation increases simultaneously after adult emergence (Calvert and Corbert, 1973), and in German cockroach *Blattella germanica* (Schal et al., 1997) and armyworm moth *Pseudaletia unipuncta* (Cusson and McNeil, 1989) both these processes were controlled by Juvenile Hormone. Hormonal levels are affected by female nutrition and in *Heliothis virescens,* for example, nutrient availability affects the hormonal precursor for egg maturation and pheromone production resulting in an inverse relationship between them (Foster et al., 2014). Therefore, it is plausible that pheromone quantity and quality vary with female fecundity in *E. kuehniella* and that this is detected by males.

In the black field cricket *Teleogryllus commodus*, Hunt et al. (2005) found that lighter females at high diet conditions are less sexually responsive compared to heavier females and attributed this to variation in resource acquisition. Likewise, fast growing nymphs of *Drosophila melanogaster* are light due to lower resource acquisition and assimilation capacity and thus in poor condition (Chippindale et al., 1997). In some butterflies, slow developing larvae were small, took longer to mate and produced fewer sperm (Lewis et al., 2010). In the present study, a lack of difference in the reproductive success between males at low density (200) and high density (800) indicates poor condition/genetic constitution of former under *ad libitum.*

Contrary to the expectation that only males in good condition can perform intense courtship, it was found that male *E. kuehniella* that developed at low population density (200) courted females for longer (Table 2). This may be because males that perform intense courtship had greater mating success. In *Drosophila grimshawi* males that displayed courtship longer were preferred by females as mating partners (Droney, 2003). Likewise, in the moth *Ostrinia scapulalis*, male courtship activity and mating success were positively correlated (Win et al., 2013). However, sexual signals like courtship activity have been considered to provide honest information about male quality (Zahavi, 1975). Therefore, it is possible that female *E. kuehniella* can discriminate males based on their courtship activity. The results were consistent with observation of the European grape moth *Lobesia botrana* where females assess male quality during the pre-copulatory phase and change their behaviour accordingly (Muller et al., 2015). Likewise in arctiid moth *Utetheisa ornatrix,* when the weight of potential male mates was matched, the quantity of pheromone released during courtship was used to discriminate male quality by females (Iyengar et al., 2001).

Many studies of insects show low rates of survival to the adult stage under conditions of higher densities irrespective of the quality of individuals that survived. Mortality could be density-independent seen during early instar stage and mortality becomes density-dependent as the individuals grow and age. Density-dependent mortality is expected to have eliminated the smallest/weakest individuals probably through cannibalism, predation or pathogen attack (Wall and Begon, 1986). For *E. kuehniella* at a high experimental population density (800) and resulting food shortage, mortality was high. It is possible that at higher population density (800) as the competition for food sources increases mortality could be size-dependent.

In a benign environment it is possible that most mortality is random with respect to genotypic variation so that all genotypes have an equal likelihood of survival. In a large population the likelihood of genetic drift influencing genotypic composition is low. In contrast, harsh environments can result in high mortality, that may be non-random, so that in stressed conditions individuals with appropriate genotypes have a higher chance of survival (Bussiere et al., 2008). For example, in the barnacle *Balanus balanoides* it was found that regardless of size all individuals survived at low and intermediate densities, whereas at high density survival was density dependent and smaller individuals had greater mortality rates compared to large individuals (Wethey, 1983). Likewise in plants, as populations become crowded with age and growth, thin plants are eliminated (Ford, 1975; Hiroi and Monsi, 1966). This indicates that individuals that survive at high population density (800) have the better ability to resist to stress, fight against pathogens etc. Survival of *E. kuehniella* larvae under such circumstances might reflect selection for appropriate “good” genetics. By choosing these genetically superior individuals, both males and females may transfer alleles that enhance survival in stressed conditions to their offspring. Further work is needed to examine whether the offspring of these pairings are competitive in both normal and stressful conditions.

In conclusion, this study suggests that both male and female *E. kuehniella* can distinguish between mates using cues such as chemical, visual, etc other than body size. Both sexes prefer to mate with individuals in good condition irrespective of the rearing environment to obtain both direct and indirect genetic benefits.

CHAPTER 6

Heritability of parental body mass and its effects on offspring life-history traits in flour moth, *Ephestia kuehniella* Zeller

Abstract

Body size correlates positively with several life-history traits and reproductive success. Sexual selection theory suggests that traits that increase reproductive success are heritable. In the present study using the model insect, *Ephestia kuehniella* the heritable component of maternal and paternal body mass was examined. Offspring size phenotype is also influenced non-genetically through maternal and paternal effects. Hence, effects of parent body mass on offspring developmental period (time from egg hatching to adult emergence), growth rate and survival were assessed*.* Results reveal that body mass of both mother and father was inherited by sons and daughters indicating a genetic effect. Offspring growth rate was unaffected by paternal mass, but an increase in father body mass, resulted in increased offspring developmental period and the number of offspring surviving to adult stage decreased. In contrast, maternal body mass had a significant positive effect on the growth rate of sons and daughters. Also, as maternal body mass increased, the developmental period was reduced and was slower in sons than daughters. This could be due to the existing difference in growth rates between son and daughter. Both parents have a genetic effect on offspring body mass but only mothers had a non-genetic effect on offspring life-history traits which varied with her body mass and offspring sex indicating sexually antagonistic variation. The positive effect on daughters may be due to the strong correlation between body size and fecundity.

Introduction

Sexual selection theory suggests that traits related to reproductive success are heritable and driven by discrimination among mates. Numerous studies have shown that morphological and behavioural activities related to mating have heritable components (e.g. Davis and Landolt, 2012; Hedrick, 1988; Wedell and Tregenza, 1999). In insects, body size is one of the best correlates of reproductive success suggesting it is influential in pre-copulatory mate choice (Bonduriansky, 2001; Thornhill and Alcock, 1983) mainly because of its positive relationship with female fecundity (Honek, 1993) and male competitive ability (Bonduriansky, 2001). Body size is linked to several other life-history traits including longevity, dispersal, metabolism, stress-resistance, etc (Woodward et al., 2005 and references therein) and is thus a determinant of individual fitness (Blanckenhorn, 2009).

Body mass has been shown to be a heritable trait in many animals (e.g. arctiid moth *Utetheisa ornatrix*, Iyengar and Eisner, 1999; field cricket *Gryllus bimaculatus*, Simmons, 1987; yellow dung fly *Scathophaga stercoraria*, Simmons and Ward, 1991). The heritability values of fathers is slightly greater than mothers and is significantly higher for daughters than sons (Davis and Landolt, 2012). Large parents sire large offspring that have greater reproductive success (e.g. *Telostylinus angusticollis*, Bonduriansky and Head, 2007; *Cydia pomonella*, Davis and Landolt, 2012), but large offspring take longer to develop (Roff, 1992; Stearns, 1992) and have lower survival rates (e.g. Blanckenhorn, 2009; Simmons and Ward, 1991). On the other hand, growth rates are assumed to be fixed but are influenced by external factors. For example, a higher feeding rate requires more searching for food that exposes individuals to predation and thereby increased risk of mortality (Roff, 1992; Stearns, 1992).

Parents can affect the performance of their progeny through non-genetic inheritance (Bonduriansky and Day, 2009). Female gametes are large and contribute extra-nuclear material to the embryos (Trivers, 1972) that influences offspring early development, growth rate and body size (e.g. Fox, 1994; Fox et al., 1997; Heath et al., 1999; Jann and Ward, 1999; Marshall and Bolton, 2007) and are categorised as maternal effects (Mousseau and Fox, 1998).

Fathers can also exert influence on progeny development non-genetically through variations in sperm morphology and ejaculate substances (Bonduriansky and Day, 2009; Crean and Bonduriansky, 2014). Paternal effects can be mediated through changes in egg size and composition in two ways (Crean and Bonduriansky, 2014). Males can transfer nutrients that are directly incorporated into eggs and so provide developing embryos with additional nutrients (e.g. Weigensberg et al., 1998). Alternatively, accessory gland products in male ejaculates can in some circumstances modify maternal physiology and so she increases her reproductive investment in each egg resulting in a non-genetic effect on progeny fitness (e.g. Bonduriansky and Head, 2007; Gilbert et al., 2012). Despite this type of differential maternal allocation (Wedell and Karlsson, 2003), information on how parental body mass can influence offspring phenotype and fitness is still lacking (Crean and Bonduriansky, 2014).

The present study investigated whether offspring inherit their parent's body mass and to what extent parents can influence the survival, developmental period and growth rate of their offspring. Here, a single experiment over two generations was used to test the hypotheses that parental body mass is a heritable trait. Parental body mass positively correlated with offspring growth rate and reduced developmental period and increased survival.

Materials and methods

Study species

The present study reports results from the Mediterranean flour moth, *Ephestia kuehniella,* which feed only in the larval stage (Hill, 2002). In an optimal rearing environment, larvae complete their development and emerge as adults in 35–40 days with 70% adult survival rate (Bhavanam et al., 2012). Adults mature sexually within 12 h of their emergence (Xu et al., 2008) and prefer large individuals as reproductive partners (Xu and Wang, 2009a). Large females are more fecund and large males transfer larger spermatophores with more number to heavy females (Xu and Wang, 2009a). Male ejaculate typically consists of eupyrene and apyrene sperm and accessory gland products. Offspring from large parents are big (Xu and Wang, 2013) but it is not known how paternal body mass influences the offspring life-history traits during development (growth rate, developmental period and survival) or its heritability estimates.

Laboratory stock culture at a rate of 100 larvae was maintained on 50 g of diet (43.5% wholemeal wheat flour, 43.5% maize meal, 3% brewer's yeast and 10% glycerine by weight) (Lima-Filho et al., 2001). A paper towel (25 cm × 25 cm) was placed in each container for pupation. Insects were maintained at a temperature of 25 ± 1°C, 60 ± 10% relative humidity and 14:10 h (light:dark) cycle.

Mature pupae were gathered, weighed using an electronic dual range balance (Mettler AE100, Mettler-Toledo Inc., Switzerland) with 0.1 mg precision, and placed in separate glass vials (2 cm diameter × 7.5 cm high). Mass changes due to water loss were minimized by using only adults that emerged within 12 h of mass measurement. In the first scotophase after adult emergence, 144 one day old virgin males and females were randomly selected, paired and allowed to mate in separate plastic containers. After copulation, males were removed and the females were left to oviposit in the same containers. To avoid confounding effects of parental mating history and age on offspring fitness, eggs laid by each female within 24 h of first mating were collected into separate Petri dishes (8.5 cm diameter), incubated for three days and used for the experiment. Upon hatching, 50 neonate larvae less than 12 h old laid by each female were randomly selected and transferred to a plastic container (8 cm diameter × 10 cm high) that was filled with 25 g of the above diet. Similar procedure was repeated for all females establishing 72 replicates with different parent body mass combinations.

Body mass heritability

In *E. kuehniella*, there is a strong correlation between pupal mass and adult body mass (Xu and Wang, 2009a) and hence, pupal mass was used an estimate of adult body mass. To determine whether parental body mass is heritable, from each replicate 20 mature pupae (10 sons and 10 daughters) were collected randomly weighed as above and each placed separately in a glass vial. For each replicate, the mean body mass of sons and daughters were calculated separately and were then regressed against their respective values of the father and mother body mass. For single-parent-offspring regression, heritability values were obtained by multiplying the slope of each regression by 2. Standard errors were doubled (Falconer, 1989; Iyengar and Eisner, 1999). The slope of the regression line is considered to be equivalent to the heritability estimate for mid-parent-offspring regression.

Influence of parent body mass on offspring developmental period, growth rate and survival

After 20 days of larval rearing, each rearing container was checked for adult emergence every second day until all surviving pupae had developed into adults to determine the average survival rate (total number of adults emerged/total number of neonate larvae assigned). From each replicate, 10 pupae of each sex were randomly selected and their larval period (number of days from hatching to pupation), developmental period (days from hatching to adult emergence), pupal mass and growth rate calculated as (ln (pupal mass)/larval period and multiplied by 100) was recorded.

A generalized linear mixed model (GLMM) was used to test for paternal and maternal effects on offspring developmental period and growth rate. Mother and father body mass were included as fixed effects and family as random effect, fitted by REML (residual maximum likelihood). The initial model consisted of fixed effects and the interaction between them. If the interaction effect was non-significant, it was removed and the model rerun. Survival rate (%) was analysed using GLM with binomial distribution with logit link function. The formula cbind (sur, act-sur) was a two-column response matrix, where the sur is the number of surviving offspring and act is the initial number of larvae placed in the container at the start of the experiment. All the statistical analyses were carried out with R (R

Core Team) using nlme package (Pinheiro et al., 2015) and 3D surface graphs were plotted using lattice package (Sarkar, 2008).

Results

Heritability estimates

Mean body mass of daughter and son was 25.87 ± 0.21 mg and 22.97 ± 0.17 mg, respectively. Parent body mass was significantly heritable to both sons (father: $F_{1,70}$ = 15.45, P = 0.0002; mother: $F_{1,70}$ = 13.26, P = 0.0005) and daughters (father: $F_{1,69}$ = 16.98, P = 0.0002; mother: $F_{1,69}$ = 12.09, P = 0.0009) (Table 1, Figure 1).

Table 1. Heritability estimates (h^2±SE) and correlation coefficient (r) for parent-offspring body mass in *Ephestia kuehniella*

	Son		Daughter	
	h^2 ± SE.	r	h^2 ± SE	r
Mother	0.36 ± 0.10	0.40	0.42 ± 0.12	0.39
Father	0.50 ± 0.12	0.43	0.64 ± 0.14	0.44
Mid-parent	0.37 ± 0.07	0.54	0.45 ± 0.08	0.55

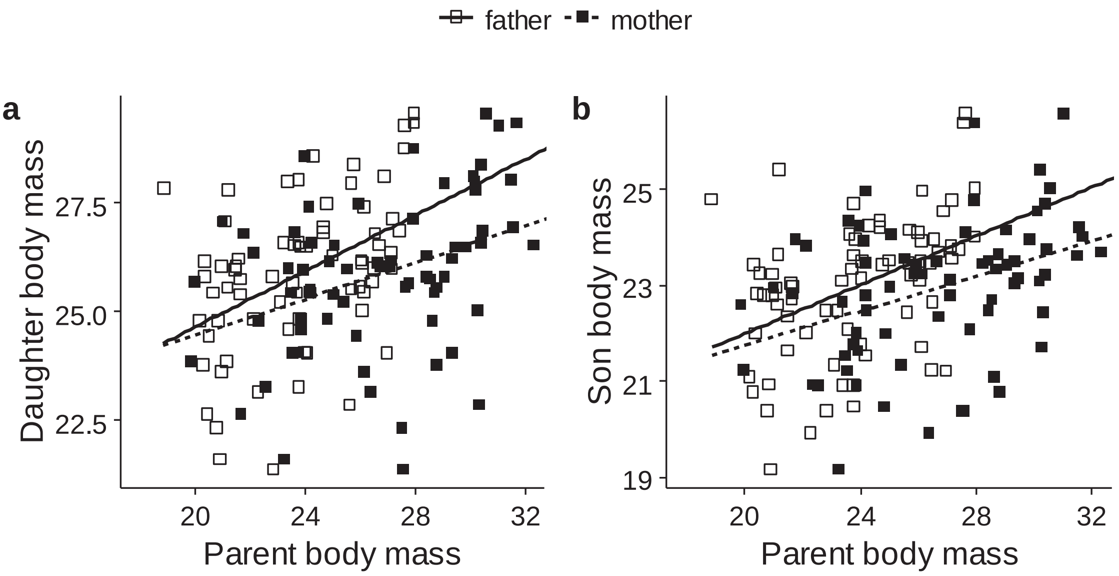

Figure 1. Regression of mean daughter (a) and son (b) body mass (mg) on parent body mass (mg) for *Ephestia kuehniella*. The heritability estimates of single parent-offspring regression and mid-parent-offspring regression were significantly high for each offspring sex (Table 1).

Maternal and paternal effect on offspring

Developmental period

There was a significant mother and father interaction on the developmental period of daughters (t = —2.13, P = 0.037) but not sons (t = —1.56, P = 0.123) (Table 2, Figure 2a & b). At lower body mass mothers had less effect on daughter developmental period than did fathers, but the maternal effect on developmental period increased more quickly with body mass. Both the mother and father had a significant positive effect on daughter (mother: t = 1.96, P = 0.05; father: t = 2.25, P = 0.03) developmental period. After removal of the interaction term, son developmental period was not affected by father body mass (t = 1.85, P = 0.068) and mother body mass (t = —2.27, P = 0.027), suggesting that those with heavier body mass had a stronger effect on developmental period.

Growth rate

Maternal body mass had a significant effect on the growth rates of sons (t = 3.27, P = 0.002) and daughters (t = 2.96, P = 0.004). Growth rate of each offspring sex increased with the increase of mother body mass (Table 2, Figure 2c & d). Neither the son's (t = 0.24, P = 0.808) nor the daughter's (t = 0.31, P = 0.754) growth rate was influenced by father body mass.

Survival rate (%)

Survival rate was significantly affected by father (z = —3.97, P < 0.0001) but not mother (z = —0.062, P = 0.951) body mass (Table 2, Figure 2e). This suggests that with increase in father body mass the offspring survival rate decreased.

Table 2. Estimates obtained using generalized linear mixed model and general linear model with binomial distribution that explains the maternal and paternal effects on offspring developmental period, growth rate and survival in *Ephestia kuehniella*. Degrees of freedom were 1 for all terms.

	Developmental period		Growth rate		Survival rate
	Daughter	Son	Daughter	Son	
Intercept	4.02	36.06	10.42	9.79	1.52
Mother body mass	1.07	—0.13	0.07	0.07	—0.0006
Father body mass	1.43	0.13	0.009	0.007	—0.0558
Mother body mass * Father body mass	—0.05				

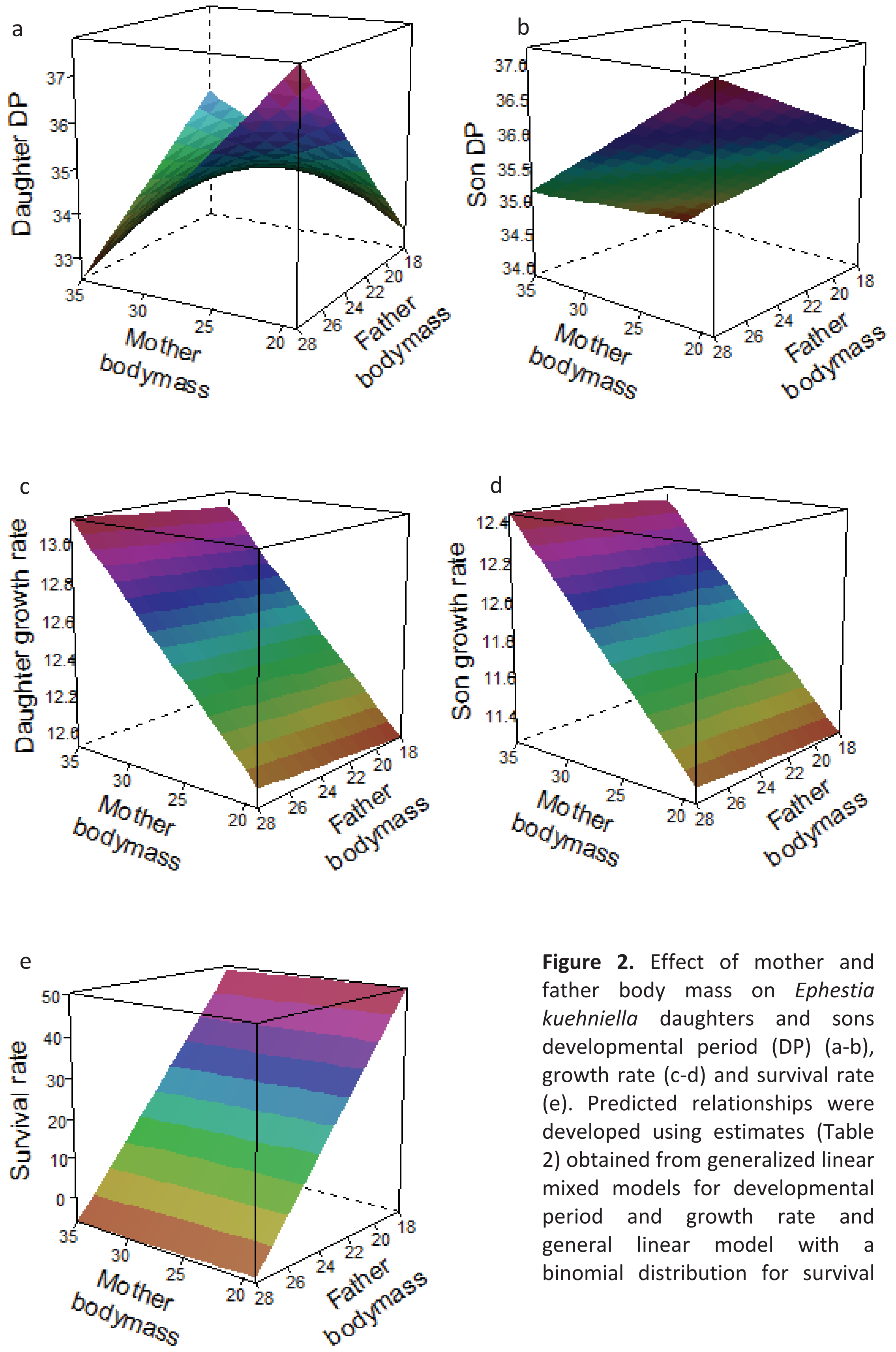

Figure 2. Effect of mother and father body mass on *Ephestia kuehniella* daughters and sons developmental period (DP) (a-b), growth rate (c-d) and survival rate (e). Predicted relationships were developed using estimates (Table 2) obtained from generalized linear mixed models for developmental period and growth rate and general linear model with a binomial distribution for survival

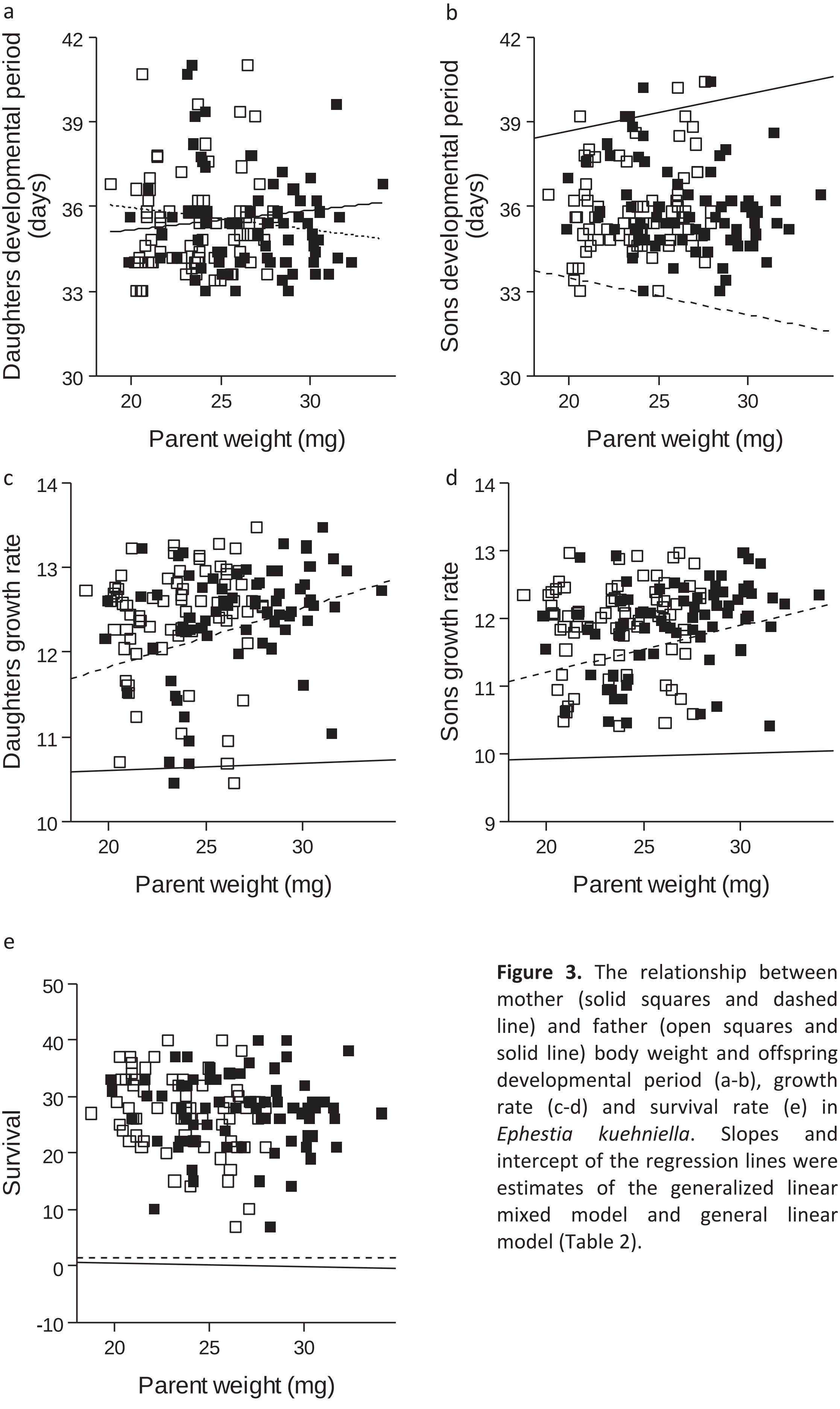

Figure 3. The relationship between mother (solid squares and dashed line) and father (open squares and solid line) body weight and offspring developmental period (a-b), growth rate (c-d) and survival rate (e) in *Ephestia kuehniella*. Slopes and intercept of the regression lines were estimates of the generalized linear mixed model and general linear model (Table 2).

Discussion

Parent body mass is heritable in moths (Davis and Landolt, 2012; Iyengar and Eisner, 1999), crickets (Simmons, 1987) and flies (Simmons and Ward, 1991). In addition, using arctiid moth *Utetheisa ornatrix,* Iyengar and Eisner (1999) suggested that male spermatophore size had no effect on heritable component of body size. As with other studies in *E. kuehniella* sons and daughters inherited their parents' body mass and were correlated as predicted (Table 1, Figure 1). This indicates that offspring body size is genetically controlled. The present results also highlight the benefits obtained from pre-copulatory mate choice. By choosing large partners not only do males obtain higher fecundity returns and females receive large spermatophores but they are also highly likely to gain genetic benefits. Offspring that inherit their parent's body mass will have greater reproductive success due to positive correlation between body size and reproduction.

In insects, body mass is obtained through increased developmental rate or growth rate and/or egg size (Blanckenhorn, 2000). Besides the genetic component, parents can have a non-genetic effect on offspring development, growth and survival. Maternal effects are mediated through nutrients provisioned in eggs (Mousseau and Fox, 1998) and reported in many species of plants (Riginos et al., 2007), fish (Mogensen and Hutchings, 2012), reptiles (Warner and Shine, 2007), birds (Neff and Lister, 2007), insects (Jann and Ward, 1999) and mammals (Skibiel et al., 2009). Similarly, paternal effects involve factors including sperm, accessory gland products, differential maternal allocation, nuptial gifts and nutrients that are invested in egg (Bonduriansky and Day, 2009; Crean and Bonduriansky, 2014).

In *E. kuehniella*, there was a significant influence of father body mass on developmental period (Figure 2a & b) and survival rate (Figure 2e) but not on growth rate (Figure 2c & d). On the other hand, there was a significant effect of mother body mass on growth rate of sons and daughters (offspring produced by large mothers had higher growth rates) (Figure 2c & d). In addition, a significant first order interaction between mother and father body mass on daughter developmental period (Figure 2a) but not son developmental period (Figure 2b) was detected. This interaction resulted from the negative mother body mass on daughter developmental period; daughters produced by large mothers had shorter developmental period indicating a non-genetic effect of mother on developmental period.

The observed maternal effect on daughter developmental period is likely to be due to differences in egg provisioning. Large mothers deposit high quantity and quality nutrients into their eggs. Eggs with high yolk quality have greater yolk conversion efficiencies and absorption rates, and large larvae emerge from the egg (Campbell, 1962; Rossiter, 1991). Large larvae are highly mobile and have higher feeding rates, and as a result these offspring achieve the fixed body size (genetically-determined due to parental body mass heritability) more quickly. So, offspring developed from large eggs and large mothers have shorter development period compared to offspring from small eggs and small mothers.

Fathers can influence the offspring developmental period in two ways (Crean and Bonduriansky, 2014): direct investment of nutrients in the eggs. In some hemiptera, male investments were incorporated into the eggs that increase the egg size (McLain and Pratt, 1999). In crickets, it was shown that male body size determined the amount of nutrients provisioned in each egg (Paczolt and Jones, 2010; Weigensberg et al., 1998). In *E. kuehnialla* males transfer a spermatophore during mating and its size depends on the body mass of both male and female (Xu and Wang, 2009a). However, females of this species do not seem to use male resources for egg production (Xu and Wang, 2011).

Paternal effects on offspring development are mediated through differential maternal allocation. Females of some species use differences in male phenotype as a cue to alter investments in eggs (Wedell and Karlsson, 2003). In addition, in *Drosophila melanogaster* the physiological act of mating (Priest et al., 2008), and in *Cheilomenes sexmaculata* longer copulation duration (Mirhosseini et al., 2014), stimulated females to produce more offspring with greater investment in each offspring. In *E. kuehnialla* the process of egg chorination is completed soon after adult emergence and before mating (Calvert and Corbert, 1973) eliminating the possibility of increased resource allocation in eggs based on mating and male size. This explains why father body mass does not have an influence on offspring growth rate, and developmental period is consequently increased and survival decreased. This study reveals absence of non-genetic paternal effects on offspring in *E. kuehniella*.

There is a trade-off between body size and developmental period in *E. kuehniella*. Offspring developmental period increased and survival rate decreased with increased paternal body mass (Figure 2). Similar results have been reported in grasshoppers (Wall and Begon, 1986) and beetles (Bonduriansky and Head, 2007). Increased developmental period increases risk of mortality (Roff, 1992), and suggests that natural selection favours a shorter developmental period because:

(1) food is exhausted with time; (2) extended developmental period increases generation time and in species that breed continuously the number of offspring contributed in each generation is decreased; and (3) overlapping generations alters the selection process in normal situations.

In the present study, it was also demonstrated that mothers affect offspring development in a sex-specific manner (absence of interaction effect on son developmental period). Female fitness is affected by body size to a greater extent than males. In females, there is a positive relationship between body size and fecundity (Honek, 1993) so the positive correlation between female body size and egg size and in turn hatch rate, survival from hatching to adult stage, growth rate and body size (McGinley et al., 1987), mothers may have greater reproductive fitness through increased daughter reproductive success. Therefore, selection might favour investment of more resources in daughters relative to sons by large mothers. At the moment it is still unknown whether *E. kuehniella* females are able to alter their investment in eggs based on the offspring sex and allocate more nutrients to daughter than son or just physiological differences of male and female larvae that interact with genotype.

In males, although large body size increases mating and/or fertilization success (e.g. Bissoondath and Wiklund, 1996), the developmental mechanisms involved to achieve greater body mass counterbalance the advantages accrued through sexual selection (Andersson, 1994). For example, in crickets, male mating success is a heritable trait but sons of successful fathers developed slowly indicating a cost of natural selection on sexual selected traits (Wedell and Tregenza, 1999). A similar trade-off occurs between male mating success and developmental period in

Drosophila robusta (Etges, 1996) and the web-building spider *Stegodyphus lineatus* (Maklakov et al., 2004). Whether males can fully compensate for this through increased male mating success is still unknown.

In summary, this work on *E. kuehniella* provides evidence of genetic and non-genetic effects of parental body mass on offspring life-history traits. Both mother and father transmit their body mass to their offspring. Offspring developmental period and growth rate were affected by mother body mass due to differences in egg provisioning that differed with mother body mass and offspring sex that indicated presence of maternal non-genetic effects. Due to the absence of direct nutrient contribution to offspring or maternal differential resource allocation by fathers, paternal effects were observed on survival. This study indicates that paternal effects were predominantly genetic whereas the mothers have both a genetic and non-genetic effect on offspring life-history traits involving an important trade-off between body size and developmental period.

CHAPTER 7

Does polyandry provide genetic benefits?

Abstract

The occurrence of polyandry in species where females do not obtain direct (material) benefits is assumed to be for indirect genetic benefits that increase offspring fitness. Here, I tested this hypothesis using the Mediterranean flour moth, *Ephestia kuehniella.* Females were mated either with the same (monandrous) or different (polyandrous) males and the neonate larvae were reared at a population density of 5, 20 or 40 individuals on constant amount of food until adult emergence for the first generation. The adults from each of the three population densities were used to develop monandrous, polyandrous and cross culture (cross between monandrous and polyandrous) clutches for the second generation and their neonate larvae were reared at the same population density as their parents. I compared various offspring life-history traits (developmental period, pupal mass, survival rate, female fecundity and hatching success) of monandrous, polyandrous and cross culture clutches. The benefits of polyandry first appeared in the second generation. Pupal mass of male and female moths was greater in polyandrous and cross culture clutches compared to monandrous clutches. Furthermore, this effect was observed only at higher population density (40). Our results suggest that through multiple mating with different males, females increase the genetic variation among the clutches which is only advantageous in a fluctuating or stressful environment.

Introduction

Polyandry is a taxonomically widespread reproductive strategy (Birkhead and Moller, 1993) whereby a single female mates with several males during its reproductive lifetime (Thornhill and Alcock, 1983). The fact that this strategy is adopted by females of a variety of animal species indicates strong fitness advantages that have evolutionary importance (Snook, 2014). Engaging in polyandry provides females of many species with increased amounts of nutritious seminal fluid, nuptial gifts, sperm, physical stimulation and larger territory that could increase a female's reproductive output and survival (Arnqvist and Nilsson, 2000). In species where no direct benefits are obtained, polyandry is suggested to increase offspring fitness through indirect genetic benefits obtained in the form of good, compatible or allelically diverse genes (reviewed by Fedorka and Mousseau, 2002; Jennions and Petrie, 2000; Slatyer et al., 2012; Zeh and Zeh, 2003); each could explain the majority of genetic benefits obtained through polyandry (McLeod and Marshall, 2009; Simmons, 2005). However, the indirect genetic benefits obtained by females that mate with different males are still unclear (Birkhead, 2000).

According to the good gene theory, polyandrous females increase their offspring viability or attractiveness by securing paternal good genes through cryptic choice or active sperm selection processes (Eberhard, 1996; Gasparini and Pilastro, 2011). The genetic compatibility theory argues that polyandry increases the probability of a female obtaining genes that are compatible with her genome and so increases net fecundity by reducing embryo mortality (Zeh and Zeh, 1997). The genetic diversity theory proposes that a female mating with several dissimilar males increases genetic variance within her brood clutch (Hughes et al., 2008). This could be advantageous for coping with changeable environmental conditions (Barbosa et al., 2012; Gillespie, 1974), it allows production of novel genotypes (Williams, 1975). Mixed matings have high probability of producing some successful offspring adapted to a given set of environmental conditions. Natural selection can lead to adaptive shift of the trait average in a population in response to environment pressures (Fisher, 1930) while higher genotypic variance may provide net benefit in fluctuating conditions, low variance may be advantageous in constant conditions.

Genetic variance within a clutch could also increase the average productivity, growth and weight of a set of individuals due to more varied resource use (Yasui, 1998) that result in less competition among each other (Young, 1981), for example, plants (Bell, 1991; Cheplick and Kane, 2004), insects (Harano, 2011; Mattila and Seeley, 2007) and marine animals (Greenberg et al., 2002). In contrast, individuals with similar genotypes may compete more intensively for resources and thus have lower net population productivity. Therefore, competition in less diverse groups may increase selection pressure on homogenous populations even in tautology conditions. In the reverse scenario, genetically diverse populations in adverse conditions (Agashe and Bolnick, 2010; Martin et al., 1988) have faster rates of evolutionary response (Ayala, 1965) because there is a mechanism for replacement of the existing genotype (Fox and Rauter, 2003). Although genetic diversity has clear evolutionary and ecological importance (Johnson and Brockmann, 2010; Power and Holman, 2014), in the past decade studies to determine the benefits of genetic diversity have declined (Hettyey et al., 2010) and evidence for genetic diversity being beneficial is still weak in studies of insects (Snook, 2014).

Here, the Mediterranean flour moth, *Ephestia kuehniella*, was used as a model insect to consider polyandry. As female fecundity and male mating success decreases with age, males and females remate at shorter intervals (Xu and Wang, 2009a). Adult females lay up to 300 eggs in close vicinity (chapter 2), the eggs hatch synchronously and the neonate larvae do not move. This is significant because developing larvae feed at the site of hatching, and so may be exposed to nutrient limitation, crowding and high sibling contact. Due to low dispersal rates (Rees and Rangsi, 2004), it is likely that offspring mate with close relatives for many generations, so polyandry could serve as a mechanism to increase genetic diversity and relative fitness of broods.

This prediction was tested by comparing the rates of larval survival, developmental period, pupal mass and female fecundity and hatching success among monandrous, polyandrous and cross culture clutches. If polyandry enhances offspring performance then polyandrous and cross culture clutches will have a greater survival rates, shorter developmental period and heavier pupal mass than monandrous clutches. Effects of genetic diversity are expected to be more prominent in stressful conditions (Holman and Kokko, 2013), with polyandrous and cross culture clutches better able to adapt to stressful conditions or show superior performance in these conditions.

Materials and methods

Two separate colonies of *E. kuehniella* (A & B) were reared in captivity for six generations prior to the experiments. Larvae were maintained at a density of 100 per container on 50 g of standard diet (43.5% wholemeal wheat flour, 43.5% maize meal, 3% brewer's yeast and 10% glycerine by weight (Lima-Filho et al., 2001) in plastic containers (8 cm diameter × 10 cm high), at 25 ± 1°C, 60 ± 10% relative humidity and 14:10 h (light:dark) cycle.

Mating procedure

Mature pupae were collected from colony A, weighed using an electronic dual range balance (Mettler AE100, Mettler-Toledo Inc., Switzerland) with an accuracy of 0.1 mg and kept in separate glass vials (2 cm in diameter × 7.5 cm in high) until adult emergence. Pupal mass was categorized as average, light (> 1SD below the mean) or heavy (> 1 SD above the mean). Average pupal mass for males was 23.02 ± 0.48 and females 25.29 ± 0.62 mg, which is typical for this species. In the first scotophase, 30 one day old, average size, virgin females and males were selected at random. Adults were paired and allowed to mate in plastic containers (6 cm in diameter × 8 cm in high). After mating, females were left in the same containers and males were returned to their glass vials. In the following scotophase, females were again presented with males. Half (15) were given the same male from the previous scotophase, and the remaining 15 females were provided with a new male from a different lineage, colony B. The new males were 2 days old and had previously mated once before being introduced to 15 females from colony A. This pre-mating was done to minimize confounding effects of male age and mating history on the treatment female. Thus all second mates for females had themselves mated once before. All pairs were observed for mating and only twice-mated females were considered for the experiment. Thus two treatments, monandry and polyandry with 15 and 11 replicates respectively, were established. After copulation, males were removed from the containers and females were left to oviposit in the same container. Eggs laid by each female were collected daily into separate Petri dishes (8.5 x 1.5 cm diameter) until she died. Females that mated twice with the same male or with different males laid an average of 260.62 ± 10.65 and 256.78 ± 9.84 eggs, respectively, these values being not differing significantly ($F_{1,24}$ = 1.15, P = 0.295). Three days after incubation, eggs were observed for hatching and the total numbers of neonate larvae were counted. There was no significant difference in the hatching success of monandrous and polyandrous females (monandrous: 97.65 ± 0.66%; polyandrous: 98.45 ± 0.50%) ($F_{1,24}$ = 2.10, P = 0.16).

Procedure to establish first & second offspring generations

Offspring of monandrous and polyandrous females are referred to as MF and PF, respectively. In the first generation, neonate larvae (< 12 h old) of monandrous and polyandrous females were reared in glass vials (2 cm in diameter × 7.5 cm high) containing a diet of 2.5 g at population densities of 5, 20 or 40. Thus, six treatments were established for the first generation. There were fifteen replicates of each MF treatment and eleven replicates of each PF treatment.

The second generation was developed using first generation adults. In the first scotophase, from each replicate of MF-5, PF-5, MF-20, PF-20, MF-40 and PF-40 treatments, we paired randomly selected average weight, virgin males and females of one day age in plastic containers. In addition, cross culture treatments (CC-5, CC-20 and CC-40) were obtained by pairing randomly selected, one day old average virgin MF females/males and PF males/females from different colonies. In the second scotophase, eggs were collected from each female and placed in separate Petri dishes and incubated for 3 days. Upon hatching, neonate larvae (< 12 h old) from each mother were reared at the same density as their respective mother's until adult emergence (Figure 1). Thus, nine second generation treatments were produced: MF-5, n = 5; MF-20, n = 10; MF-40, n = 10; PF-5, n = 6; PF-20, n = 7; PF-40, n = 7; CC-5, n = 7; CC-20, n= 15; and CC-40, n = 18. Unsynchronized adult emerging dates resulted in unequal numbers of replicates.

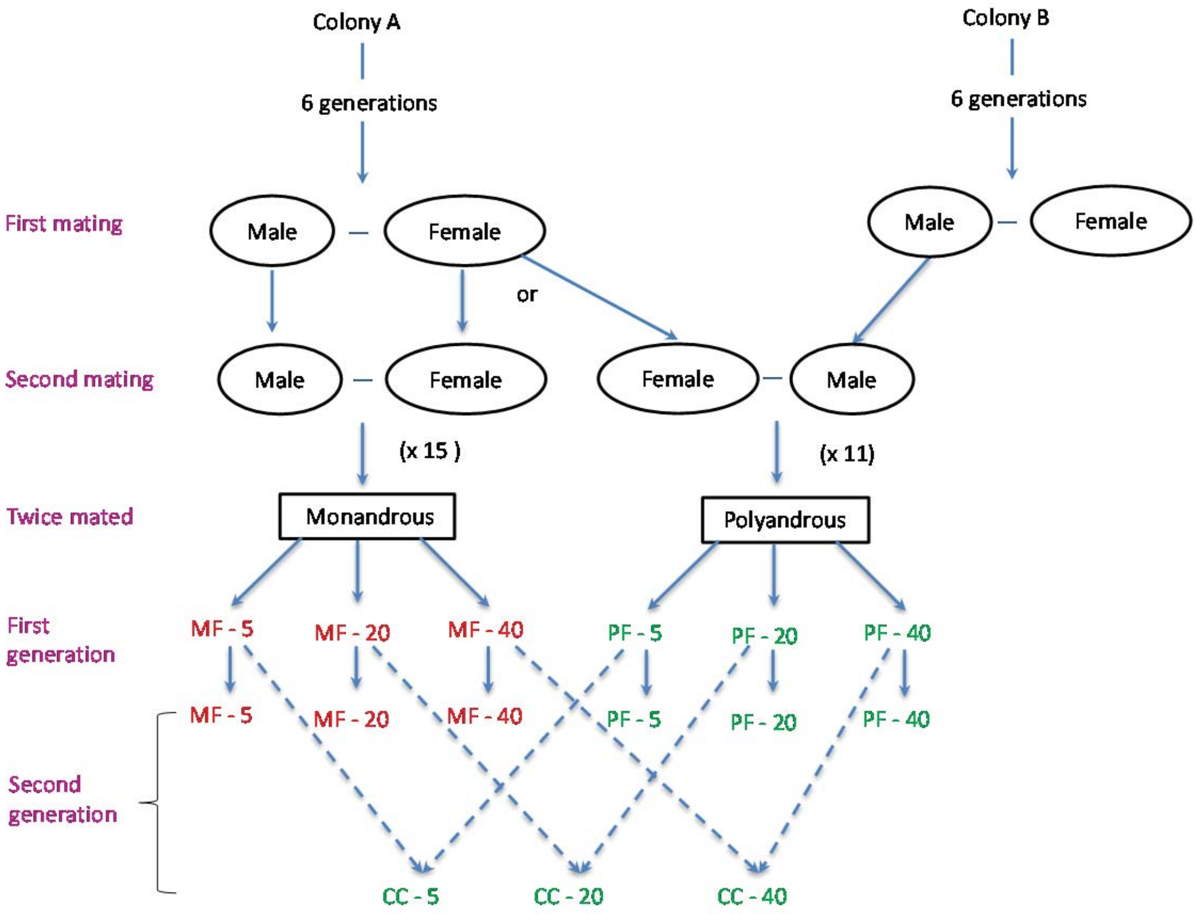

Figure 1. Schematic diagram mating and rearing procedures used to examine effects of polyandry. On two consecutive days, females were allowed to mate twice with the same (monandrous (MF)) or different males (polyandrous (PF)). From each female, eggs laid after second mating were collected and upon hatching neonate larvae were split and assigned to one of the three rearing densities (5, 20 and 40), thus in the first generation six treatments (MF-5, MF-20, MF-40, PF-5, PF-20 and PF-40) were established. Adults of the first generation were allowed to mate once. Solid lines indicate mating within the treatment and dashed lines indicate mating between MF and PF adults that belong to the same population density and hence called cross culture (CC). Neonate larvae of these matings were used to develop second generation.

Offspring larval performance (percent survival, developmental period and pupal mass)

To determine the effect of polyandry on offspring larval performance in the first and second generations, the developmental period (from egg hatching until adult emergence), survival rate (total number of adults emerged/total number of neonate larvae placed as a percentage) and pupal mass were recorded for each replicate. Mature pupae were placed in separate glass vials and sexed upon emergence of adults.

First generation offspring female fecundity

To determine female fecundity, I paired randomly selected 1 day old, average, virgin males and females from each treatment in a plastic container for their lifetime. Eggs were collected daily from each pair and maintained separately until the female died. For each female, the number of eggs laid was counted under a dissecting microscope (Olympus SZ III, Japan) and lifetime fecundity was determined. Incubated eggs from each pair were observed for hatching from 3rd day of incubation (egg period lasts 4-5 days) under the dissecting microscope and the numbers of eggs hatched (hatching success) was determined. Hatch rate was determined by taking the percentage of number of hatched eggs / number of eggs laid.

Statistics

Data on offspring developmental period and pupal mass were analysed using ANOVA. Multiple mean comparisons were done using Tukey's HSD test. Percentages were arcsine square root transformed before analysis. Data on survival rate (%), female fecundity, hatching success and hatch rate were not normally distributed, so these data were analysed using the non-parametric Kruskal-Wallis test followed by Dunn's procedure for multiple comparisons (Zar 1999). All statistical analyses were performed using SAS version 9.3 software with the rejection level set at $P = 0.05$. Unless and until stated, all the values reported were means ± SE.

Results

First generation

Offspring of all females (monandrous and polyandrous) reared at a population density of 40 had significantly longer developmental periods than offspring from females reared at population densities of 5 & 20 ($F_{5,71} = 20.4$, $P < 0.0001$) (Figure 2a). Consequently, survival rates were significantly lower at MF-40 and PF-40 treatments with no significant differences among the other treatments ($F_{5,66} = 3.12$, $P = 0.014$) (Figure 2b). Male ($F_{5,51} = 34.03$, $P < 0.0001$) and female ($F_{5,50} = 37.75$, $P < 0.0001$) pupae from MF-5 & PF-5 treatments were significantly heavier than those developed at higher densities (MF-20, PF-20, MF-40 & PF-40, Table 1).

Females from population densities of 20 and 40 laid significantly fewer eggs and had fewer hatching larvae than females emerged from a population density of 5, however, the number of eggs laid was not affected by mating treatment (fecundity: $F_{8,71} = 4.46$, $P = 0.0002$; hatching success: $F_{8,71} = 4.61$, $P = 0.0001$) (Table 2). There was no difference in the hatch rate of eggs among females from different density populations ($F_{8,71} = 1.54$, $P = 0.16$) (Table 2).

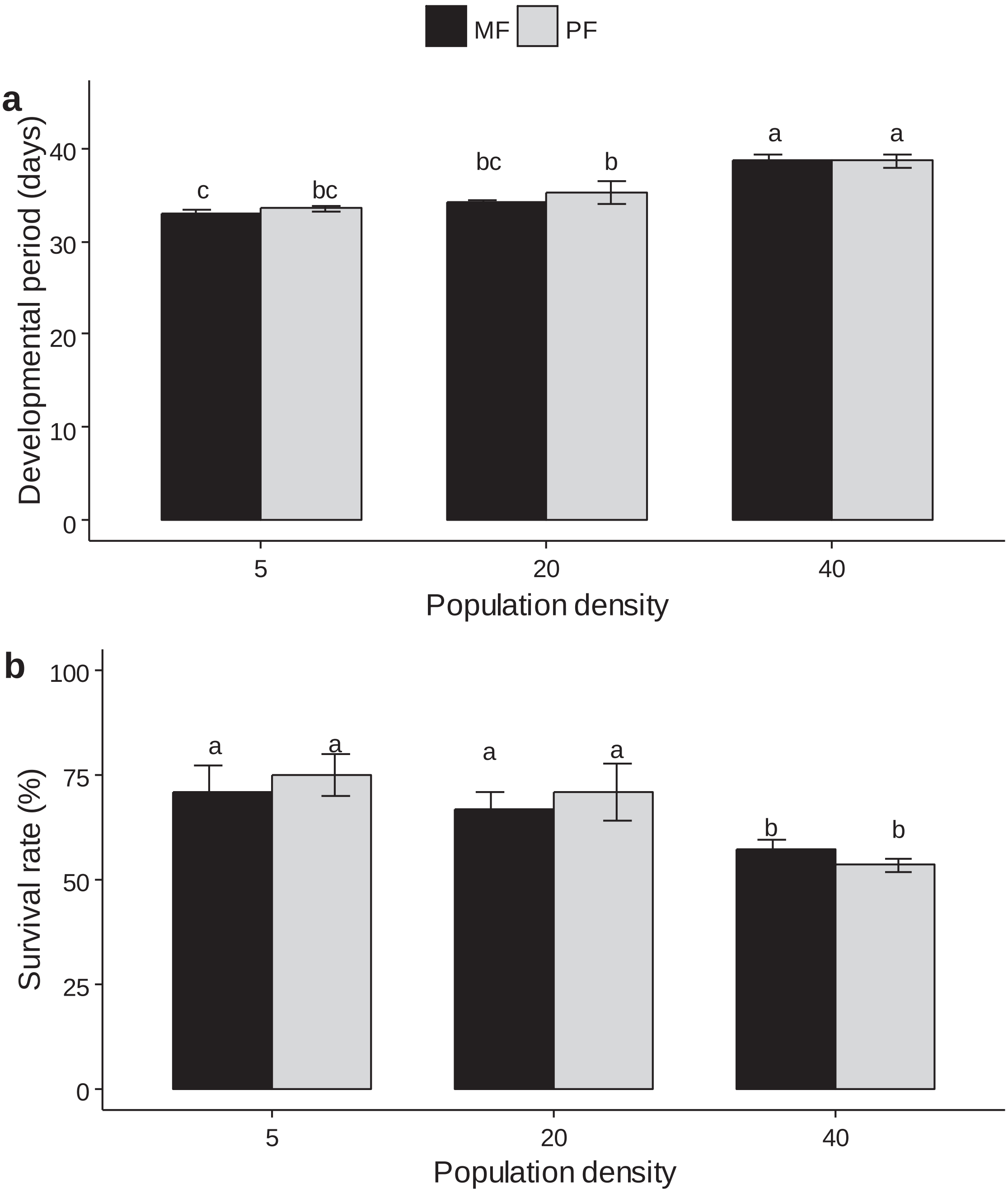

Figure 2. Mean developmental period (a) and survival (b) of first generation *Ephestia kuehniella* offspring taken from monandrous (MF) and polyandrous (PF) clutches that were reared at population densities of 5, 20 and 40. Error bars are ± SE. Different letters above each bar indicate significant difference ($P < 0.05$).

Table 1. Mean (±SE) male and female pupal mass (mg) of first and second generation *Ephestia kuehniella* offspring taken from monandrous (MF), polyandrous (PF) and cross culture (CC) clutches reared at population densities of 5, 20 and 40. Numbers within parentheses are sample sizes. In each column, means followed with different letters indicate significant difference ($P < 0.05$).

Population density	Mating treatment	First generation		Second generation	
		Male	Female	Male	Female
5	MF	24.34 ± 0.77 a (7)	26.11 ± 0.81 ab (6)	24.02 ± 0.55 a (15)	24.90 ± 0.65 ab (15)
	PF	25.07 ± 0.88 a (10)	27.01 ± 0.71 a (10)	22.72 ± 0.47 ab (15)	26.55 ± 0.42 a (15)
	CC			22.23 ± 0.59 ab (15)	25.41 ± 0.65 a (15)
20	MF	20.41 ± 0.66 b (10)	23.87 ± 0.50 b (10)	18.06 ± 0.52 cd (15)	19.47 ± 0.38 de (15)
	PF	20.92 ± 0.59 b (10)	23.38 ± 0.65 b (10)	20.55 ± 0.50 bc (15)	21.05 ± 0.80 cd (15)
	CC			21.31 ± 0.38 ab (15)	22.51 ± 0.22 bc (15)
40	MF	13.83 ± 0.66 c (10)	16.26 ± 0.59 c (10)	**11.48 ± 0.99 e (15)**	**13.40 ± 0.74 f (15)**
	PF	16.82 ± 0.80 c (10)	20.09 ± 0.68 c (10)	16.13 ± 0.95 d (15)	17.59 ± 1.15 e (13)
	CC			15.88 ± 0.76 d (15)	17.05 ± 0.55 e (15)

Table 2. Mean (±SE) fecundity (total eggs laid), hatching success and hatch rate (%) of first generation *Ephestia kuehniella* offspring taken from monandrous (MF), polyandrous (PF) and cross culture (CC) clutches reared at three different population densities (5, 20 and 40). Numbers within parentheses are sample sizes. In each column means followed by different letters are significantly different (*P* < 0.05).

	Fecundity	Hatching success	Hatch rate
MF-5	236.83 ± 30.57 ab (6)	235.33 ± 30.92 ab (6)	99.22 ± 0.55 a (6)
PF-5	300.67 ± 17.28 a (6)	295.00 ± 18.41 a (6)	97.95 ± 1.04 a (6)
CC-5	287.33 ± 21.69 a (6)	285.33 ± 21.18 a (6)	99.37 ± 0.31 a (6)
MF-20	217.08 ± 18.48 b (12)	203.67 ± 17.18 b (12)	94.44 ± 2.14 a (12)
PF-20	221.00 ± 34.53 b (7)	213.14 ± 34.16 b (7)	96.01 ± 1.31 a (7)
CC-20	214.60 ± 15.66 b (15)	206.80 ± 15.80 b (15)	96.18 ± 0.98 a (15)
MF-40	179.67 ± 14.79 b (9)	176.00 ± 13.50 b (9)	98.30 ± 0.71 a (9)
PF-40	168.00 ± 17.46 b (5)	162.80 ± 17.88 b (5)	96.72 ± 1.10 a (5)
CC-40	175.5 ± 11.65 b (14)	171.79 ± 11.97 b (14)	97.64 ± 0.68 a (14)

Second generation

In the second generation, survival rates were significantly lower in the higher density (MF-40, PF-40 and CC-40) treatments with no significant differences among the other treatments ($F_{8,76}$ = 15.20, P <0.0001) (Figure 3b). Offspring of monandrous, polyandrous and cross culture clutches reared at the highest population density (40) had significantly longer developmental period than offspring of monandrous, polyandrous and cross culture clutches reared at population densities of 5 & 20 ($F_{8,72}$ = 21.45, P <0.0001) (Figure 3a). Regardless of offspring sex, pupae from offspring of monandrous females reared at a population density of 40 were significantly lighter than pupae from offspring of polyandrous and cross culture females reared at the same population density and the pupal mass of monandrous, polyandrous and cross culture clutches reared at population density (40) was significantly lower than those reared at population densities of 5 and 20 (male: $F_{8,126}$ = 37.1, P <0.0001; female: $F_{8,124}$ = 46.16, P <0.0001) (Table 1).

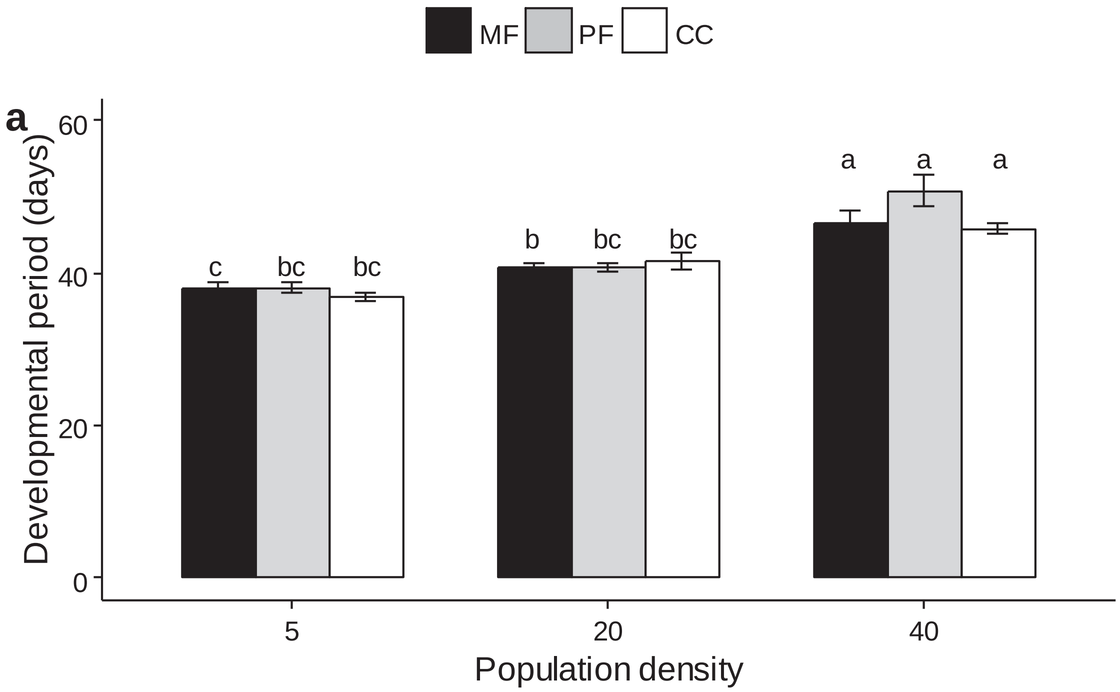

Figure 3. Mean (±SE) developmental period (a) and survival (b) of second generation *Ephestia kuehniella* offspring taken from monandrous (MF), polyandrous (PF) and cross culture (CC) clutches reared at three different population densities (5, 20 & 40). Bars with different letters indicate significant difference at P = 0.05.

Discussion

Polyandry is a reproductive strategy that could provide indirect genetic benefits in the form of good genes, compatible genes and genetic diversity that increase offspring fitness (Jennions and Petrie, 2000). Offspring survival rate and developmental period did not differ between monandrous and polyandrous females at a given density although these traits were affected by population densities (Figures 2 & 3). A similar trend was observed for female fecundity and hatching success (Table 2), and is probably a reflection of food shortage at higher population densities (Hooper et al., 2003). Under limited nutrient supply (high population density), larvae extend their developmental period until the critical weight needed for adult emergence is achieved (Bauerfeind and Fischer, 2005). An increased delay in development time and starvation at higher densities might have resulted in cannibalism (Savoldelli, 2006) and thus contributed to lower survival rates at higher densities.

A finding consistent with polyandry benefit was the effect of paternity on pupal mass. In the first generation monandrous and polyandrous clutches displayed no significant difference in pupal mass but in the second generation, offspring of monandrous females reared at high density had significantly lower pupal mass than those of polyandrous and cross culture females reared at the same density and lower density (Table 1). The appearance of a positive effect on offspring mass in polyandrous and cross culture clutches in later generations indicates a genetic effect, suggesting that genetic variation existed between full-sibs and half-sibs (Yasui, 1998). This small variation was sufficient to increase offspring success so females gained significant fitness benefits through polyandry in some circumstances (Barton and Post, 1986).

Polyandry increases the genetic variation within a population and greater genetic diversity between individuals increases the survival and size due to less competition for resources and this positive outcome was evident within a single generation in solitary ascidian *Ciona intestinalis* (Aguirre and Marshall, 2012). In contrast we observed that the second generation offspring had greater pupal mass than the first generation offspring and only at higher density (Table 1). It is known that female *E. kuehniella* that have mated with two males in a short time span use sperm from both males to fertilize the eggs and produce clutches sired by different males (Xu and Wang, 2009b).

Many studies on microbes, plants and animals show that genetic variation has a greater impact on population fitness under stressful conditions (reviewed in Wise et al., 2002) where rapid adaptive response to novel environments is favoured (reviewed in Thompson, 1998) for population persistence (Agashe, 2009). Studies on *Drosophila* flies and *Tribolium* beetles show that populations with greater genetic variation induced by mutation or hybridisation have greater productivity and adjust faster to novel environments than normal populations (Ayala, 1965; Crenshaw, 1965). Consistent with the above studies, benefits of genetic diversity were observed only under stressful conditions in the *E. kuehniella* (Table 1). At higher density, mortality is density-dependent (Wall and Begon, 1986) and as they age small individuals are at greater risk of mortality from competition or cannibalism.

The fixation of beneficial alleles is advantageous. For example, Bell (1991) found that the greater productivity of clonal mixtures of algae over monoculture was due to a sampling and selective effect of a single dominant genotype that increased productivity. If fixation of beneficial alleles occurred in the present experiment then we would expect that offspring of polyandrous females should have greater pupal mass at any density than offspring of polyandrous and cross culture females but we found no differences in pupal mass between them (Table 1). This can be explained by the experimental design, where the second generation was developed using average weight first generation individuals eliminating the heavy and low weight individuals. This study suggests that individual variation in resource use may drive the benefits of genetic diversity.

Genetic variation between individuals can increase variation in resource use, which in turn can promote niche expansion (Agashe and Bolnick, 2010). If this holds true, then the expected positive outcome of genetic diversity should be prominent in situations that create intense competition for resources. Half-siblings resulting from polyandry could be more able to partition the available resources and reduce competition (Ridley, 1993; Robinson, 1992), and thus achieve greater growth and mass. In *E. kuehniella* every developing larva marks its own space with a scent mark and conspecific larvae tend to avoid these areas. Therefore, it is possible that the low number of surviving individuals in polyandrous and cross culture clutches might have partitioned the available limited nutrients and reduced the competition among themselves. This may result in a greater availability of time and resources that could be invested in growth and development to increase body weight in plant species (Boyden et al., 2008) and in the three-spined stickleback *Gasterosteus aculeatus* (Reusch and Langefors, 2005). These results suggest that despite higher densities, individuals in polyandrous and cross culture clutches experienced lower levels of realized competition and are better able to cope under stressful conditions than individuals in monandrous clutches.

Although our results can be attributed to the effect of genetic diversity, previous studies have argued that genetic benefits in internal fertilizers are mainly due to differential maternal allocation patterns based on male quality that indirectly influences offspring fitness. Moreover, McCormick (1999) found that genetic effects can surface in offspring fitness as a result of polyandrous females receiving different types and amounts of male ejaculates that are deposited into the egg (Garcia-Gonzalez and Simmons, 2005; Simmons, 2011). These varying concentrations of male ejaculate can greatly affect the metabolic activity of the egg and embryonic development, thus increasing growth rate and ultimately body mass after hatching. However, both of these explanations are unlikely as this type of effect rarely has large fitness consequences and are expected to decrease across generations not to increase as found here.

Polyandrous females that mate with genetically superior or more compatible males can have offspring with greater survival, growth and body mass in the bruchid beetle *Callosobruchus maculatus* (Eady et al., 2000). This explanation seems unlikely in *E. kuehniella* as average weight males were used as mating partners and female choice and sperm competition were avoided. Moreover, benefits of good and compatible genes that usually occur through dominance and epistasis, respectively (Ivy, 2007; Neff and Pitcher, 2005) produce short-term benefits (Rueppell et al., 2012). Positive outcomes should be obvious in the first generation, whereas such effects emerged in the second generation (Table 1).

In conclusion, this study suggests that polyandry in *E. kuehniella* increases offspring quality. In the first generation, differences in survival, developmental period and pupal mass between monandrous and polyandrous clutches were not apparent. In the second generation however, offspring pupal mass did increase, especially under stressful conditions. This indicates that polyandry could provide long-term advantages that can help individuals cope adverse conditions.

CHAPTER 8

General Discussion

The environment is variable and stressful (Boggs, 2009). Resource allocation changes with the environment and life-history that involves alteration in morphology, physiology and behaviour and their associated activities (West-Eberhard, 2003) to increase individual fitness. This may have ecological and evolutionary consequences (Fordyce, 2006; Miner et al., 2005) and in some cases result in evolution of the population. Not all species respond to environmental variation in the same way, but the present work adds knowledge on the plastic responses exhibited by stored product pest of Lepidoptera to changes in nutrient availability.

One of the best approaches to creating food shortage is by increasing the population density by keeping the diet amount constant (Gage, 1995). From chapter 1 it is evident that the effect of environment variation can be determined a *posteriori* based on its effect on survival and reproduction. *Ephestia kuehniella* can survive well until the population density reaches 800 larvae. However, when the population density reached 400 (8 larvae for 1 g of diet), female lifetime fecundity and fertility decreased indicating that the optimum rearing density (condition) for this species is 200 population density per 50 g of diet (chapter 2).

Resource allocation decision in response to stress

Life-history traits

In species with a single feeding life-stage like *E. kuehniella*, when the foraging stage experiences food shortage its developmental period increases (chapter 2). This may be a strategy developed to obtain sufficient nutrients that can be utilized in subsequent stages. The decision when to stop feeding determines the available reserves and the body size/mass that in turn limits the space for reproductive organs (Boggs, 2009). Poor juvenile environment had a carry-over negative affect on adult stage. Individuals developed in stressful conditions had lower body mass, reproductive success and shorter adult lifespan compared to those developed in normal conditions (chapters 2 & 4).

Morphology

Allometry of body parts changes due to resource allocation changes in the pre-pupal and pupal stage (Shingleton et al., 2007). Within the closed pupal stage this may result in physiological trade-offs between traits (Emlen, 2001) due to space and/or resource restriction. These changes may affect the individual life-history and foraging decisions. In chapters 3 & 4 it was found that not all measured morphological traits were affected by food shortage. The genital traits, that is, valva and aedeagus of males and ovipositor of females were conserved. The widths of head and thorax decreased in proportion to the body mass. In short-lived species like *E. kuehniella* sturdy body is not a requirement. Moreover, because of their nocturnal nature species detect opposite sexes through olfaction and tactile cues. The allometry of wings in males and abdominal thickness in females was altered. This strategy may result in males spending more time in foraging over females, which invest more in reproduction. This difference in response may arise because of sex-specific differences in reproductive investment and mating strategies where female reproductive success is limited by egg production and that of males' increases with his mating frequency indicating that male reproductive success is also indirectly dependent on female fecundity (Bateman, 1948). This study also suggests that when individuals are exposed to a new environment multiple traits respond differently resulting in mosaic plasticity of traits (Ghalambor et al., 2007).

Plastic response and its possible consequences

Ecological consequences

A plastic response may have implications on ecological interactions through competition, predation and species coexistence and all of them can alter the population and community stability (Miner et al., 2005). In the present study the larval (foraging) stage developmental period was increased (chapter 2), which result in delayed onset of reproduction creating a time lag in the generation as a result these populations overlap with later generations. This may result in increased competition for resources between individuals of same species as well as other species that share the common resources. This competition may trigger dispersal ability that lead to changes in the biodiversity.

Evolutionary consequences

Females can influence their offspring life-history traits through egg provisioning and maternal care (Raesaenen and Kruuk, 2007). In *E. kuehniella* offspring growth rate was positively influenced by mothers that in turn have a positive effect on developmental period (chapter 6). Natural environment is spatially and temporally variable. Theory predicts that if environment is unpredictable or under poor quality females should increase their investment in each egg (Brockelman, 1975) such that females developed in variable environments have little variance in reproductive success (Einum and Fleming, 2004; McGinley et al., 1987). Moreover, increased investment in flight lowers fecundity and offspring fitness (Zera and

Denno, 1997; Zera and Harshman, 2001). In chapter 3, it was also shown that traits that have a direct influence on offspring fitness were unaltered by changes in environment. Females increase their investment in reproduction such that they contribute more offspring to the next generation.

Selection

Developmental mechanisms are predicted to constrain selection. In chapters 3 & 4 it was shown that changes in allometry of wings in males and abdominal thickness in females result in the development of novel phenotypes suggesting that traits exhibit plasticity within a short period of time which may sometimes be adaptive resulting in evolution of traits. Fitness assays between the males from normal and stressful conditions shows a decrease in fertile sperm number and longevity of food-restricted males but these males nevertheless mated a similar number of times as males developed at normal conditions (chapter 4). In dragonflies, males with a higher ratio of flight muscles to body mass have higher mating success (Marden, 2000). Small males are more agile, require less food to sustain them, have lower metabolic costs and more energy and time for sexual activities (Blanckenhorn et al., 1995). Shifts in wing morphology may however have an impact on foraging success. Alternate reproductive tactics (ART's) are more common in many insect species that arise due to differences in body size, weaponary, etc. This is because intermediate forms are less advantageous as they have to compete with large males and be successful while they cannot fit in the small male group because of their large body/trait size relative to their body mass. Hence the switch between states occurs abruptly over a small range of body size as a result few individuals with intermediate sizes are obtained resulting in ART's development (Emlen and Nijhout, 2000).

This also indicates that although trade-offs occur in food-restricted males (increased developmental period) the acquired resources are distributed equally to various behavioural and morphological adult activities. In holometabolous insects, the process of acquiring resources is more complex (Houle, 1991). The rate and efficiency of resource acquisition is dependent on behavioural and developmental pathways and once the resources are acquired metabolic pathways are important (Houle, 1991). All these processes are controlled by several genes and unlikely to be eroded (Bussiere et al., 2008). In the absence of size-assortative mating, males and females developed in food-limited conditions showed a strong preference for mating partners (chapter 5). This indicates that genetic constitution (the type of alleles individual carries) surpasses the environmental effect.

In benign conditions, the intensity of selection was weak as most of the individuals had access to resources and environment is congenial. In contrast, under stressful conditions selection intensity is likely to be strong and may result in increased frequency of alleles that are beneficial in prevailing environment (Bussiere et al., 2008). As in other studies (e.g. Byrne and Whiting, 2011; Wall and Begon, 1986), I found that polyandry increased the genetic variation in the clutches only in stressful conditions (chapter 7). Results confirmed the superiority of polyandry over monandry in stressful environmental conditions.

Mating preferences are heritable. For example, in *Drosophila* offspring inherit their competitive nature from their parents (Partridge, 1980). In *E. kuehniella*, sexually selected trait, that is, body size is inherited (chapter 6), but further study is needed to identify whether offspring produced by food-limited males are highly competitive in both normal and stressful conditions.

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