

PATTERNS OF REPRODUCTIVE ALLOCATION IN APHIDOPHAGOUS LADY BEETLES
AND THEIR RESPONSE TO VARIOUS LEVELS OF RESOURCE AVAILABILITY

by

GERMAN ANDRES VARGAS OROZCO

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Department of Entomology
College of Agriculture

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Abstract

The manner in which organisms allocate reproductive resources for reproduction is a central question with respect to life history theory. The main objectives of this research were to i) examine lifetime patterns of reproductive allocation in the lady beetles *Coleomegilla maculata* (DeGeer) and *Hippodamia convergens* (Guérin-Ménéville) (Coleoptera: Coccinellidae) while manipulating environmental conditions that affect female body size (i.e., larval food supply), ii) to study the interaction between factors underlying female body size and the resources available during reproduction, and iii) to explore the maternal effects of female size and age on the development and survival of progeny. When different size classes of females were produced and adult females were maintained with unlimited food, there were no differences in egg size across female size in *C. maculata*, but egg size increased over time in all females. In *H. convergens*, only larger females increased egg size over time, and they laid larger eggs, on average, than did small females. Maternal body size was positively correlated with the number of eggs laid per day in both species. When three size classes of females were subjected to a fluctuating food supply as adults, female size was again positively correlated with egg and daily fecundity. Whereas both species varied daily fecundity in response to adult food supply, egg size was unaffected and demonstrated a fixed pattern of change with female age and species-specific effects of maternal body size. To observe maternal effects in *H. convergens*, three female size classes were again produced and progeny were reared from three different periods of each female's reproductive life. Offspring from later oviposition days and larger females developed faster and achieved larger adult size than those reared from earlier oviposition days. Egg size showed inconsistent correlations with developmental parameters and adult progeny size, so other, more cryptic, maternal signals were inferred to signal phenotype development in progeny. A fixed program of producing faster-developing offspring that mature to larger sizes late in the oviposition cycle is adaptive for exploiting ephemeral aphid blooms that exhibit predictable dynamics of declining prey abundance and increasing competition. In the case of *H. convergens*, resource limitation during development constrained not only body size, fecundity and egg size, but also maternal ability to manipulate progeny phenotypes.

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Approved by:
Co-Major Professor
Dr. James R. Nechols

Approved by:
Co-Major Professor
Dr. J.P. Michaud

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Dedication

To my wife, Lorena Gomez; my father, Honorio Vargas and my mother Martha Orozco; because your love is my strength.

Preface

An understanding of female reproductive strategies and their allocation of resources among progeny is central to life history theory (Sinervo 1990, Wilkinson & Gibbons 2005). Since the seminal work of Smith & Fretwell (1974) it has been proposed that females face a trade-off in the size and number of their progeny, so they face the challenge of ensuring fitness either by producing fewer large, or more numerous small offspring. It is assumed that the optimal combination of progeny size and number is shaped by natural selection to optimize individual fitness (Roff 1992, Stearns 1992, Eium & Fleming 2000, Uller & Olsson 2005), and in some animals such as birds there is usually more variation in offspring number than in offspring size within individuals (Stearns 1992, Marshall & Gittleman 1994, Dixon 2000). However, observations of diverse taxa have demonstrated that offspring size may vary just as much as offspring number (Stearns 1992, Roff 1992). To cope with environmental constraints and uncertainties, some organisms may require variable developmental responses to optimize their survival (Capinera 1979, Parker & Begon 1986, Hutchings 1991, Fox & Mousseau 1998). According to Kaplan & Cooper (1984), some level of developmental plasticity in offspring size may improve performance and survival of offspring in unpredictable environments. Environmental constraints have been shown to generate variation in both number and size of offspring in amphibians (Crump 1981), fish (Hutchings 1991), reptiles (Abell 1999), and insects (Fox and Mousseau 1998). Females have also been observed to exert influence over the phenotypes developed by their offspring (Fox & Mousseau 1998). This may take the form of increasing offspring size at the expense their number, especially when conditions for progeny deteriorate (Parichy & Kaplan 1992).

Egg size can affect both developmental rate and final offspring size in many animals (Fox & Mousseau 1998). Even small changes in egg size may have dramatic consequences for life history, especially in arthropods where growth is constrained by an exoskeleton that must be molted in a series of stages (Stearns 1992). In general, offspring hatching from large eggs often have better survival in the critical early stages of the life cycle, develop faster, and mature into larger adults than those developing from smaller eggs (Fox 1994, Bernardo 1996). Generally, egg size tends to decrease with maternal age in most arthropods (Fox & Czesak 2000).

Sometimes, the advantages of larger egg or birth size only appear under conditions of environmental stress (e.g., Ng 1988). However, egg size is not the only factor affecting neonatal progeny fitness and other, more cryptic, maternal effects may also influence offspring quality (Bernardo 1996). In the soil mite, *Sancassania berlesei* (Michael), mothers face predictable deterioration of environmental conditions for later-born progeny and eggs of older females hatch later and result in immature stages that feed longer, the extended developmental period permitting achievement of greater adult size. In *S. berlesei*, egg size-independent maternal effects give later hatched offspring a better chance of competing for resources with elder siblings (Benton *et al.* 2008).

In aphidophagous lady beetles, the ephemeral nature of prey populations i.e., “boom and bust” population dynamics) is considered to be an important factor shaping the life history traits of predators, including those related to the allocation of reproductive resources (Dixon 2000). Although lady beetle species inhabit a dietary continuum ranging from highly specialized aphid-feeders to polyphagous predators, aphids typically constitute an essential food resource that optimizes development, survival and reproduction for lady beetles (Hodek & Honěk 1996). Ephemeral prey availability constitutes an environmental constraint for aphidophagous insects that could conceivably select for some phenotypic plasticity in offspring size and number, even though it has been assumed that most variation in reproductive effort will be reflected in changes in egg number rather than egg size (Stewart *et al.* 1991, Dixon & Guo 1993, Dixon 2000). However, Honěk *et al.* (2008) found substantial variation in egg size when collecting egg masses of *Coccinella septempunctata* (L.) and *Propylea quatordecimpunctata* (L.) which “[did] not conform” to the prevailing idea that “egg size is likely to be the least and clutch size to be the most variable reproductive trait” in lady beetles.

Whereas variation in offspring number by aphidophagous lady beetles has been observed in response to changing environmental conditions (Dixon & Guo 1993), egg size has been assumed to remain near some constant minimum determined by the smallest size at which first instar larvae are able to capture their first prey item (Dixon 2000, Sloggett 2008). Egg number is determined by the size of the female and the availability of food 'income' (Honěk 1993, Dixon 2000, Dixon & Agarwala 2002). However, reproductive potential is also affected by the resource 'capital' accumulated by females during immature stages (Honěk 1993, Dixon 2000) and lady beetle reproductive allocation has not been examined in the context of factors that determine

female body size (e.g., food availability during larval development), or in the context of fluctuations in income over the course of female reproductive life.

I hypothesized that phenotypic plasticity would be reflected in both egg number and egg size in response to differing levels of food availability during development and reproduction, and over the course of female reproductive life. Additionally, that maternal effects would serve to optimize fitness in the context of reproduction on aphid populations that represent 'boom and bust' cycles of prey availability, where female body size would mediate the trajectory of reproductive effort over time, and that maternal body size could potentially interact with maternal effects.

The spotted lady beetle, *Coleomegilla maculata* (DeGeer) and the convergent ladybeetle *Hippodamia convergens* (Guérin-Ménéville) are two lady beetles native to North America, where *C. maculata* is exceptionally polyphagous among aphidophagous coccinellids that can even develop exclusively on pollen (Lundgren and Weidenmann 2004), whereas *H. convergens* is likely to rely more heavily on aphids for both development and reproduction (Michaud & Qureshi 2006), even though other suboptimal diets such as moth eggs can sustain development and reproduction.

Using manipulative laboratory experiments with *C. maculata* and *H. convergens*, I studied how a primary determinant of female body size (larval food supply) and food availability during reproduction affected offspring size and number. Furthermore, I tested for possible maternal effects on offspring development by rearing offspring from different periods of female reproductive life. The three main objectives of this research correspond to the three chapters composing this dissertation respectively.

In the first chapter, I studied the effects of larval food supply, and by extension maternal body size, on trajectories of reproductive allocation using *C. maculata* and *H. convergens* during independent experiments which used the same procedure; here three different levels of female body size were produced by manipulating the availability of food during the larval stage, but unlimited food was provided during reproduction to examine the effects of factors underlying female body size on trajectories of reproductive allocation over time. During the second chapter, and again using the same two species in independent experiments, I studied the interaction of larval food supply (capital) with resource availability during the first two months of reproduction (income). Different levels of larval food supply resulted in three categories of female body size. Then, during reproduction, an alternation of food was provided in consecutive periods of deficit and excess of food. Finally,

during the third and last chapter I tested for possible maternal effects on offspring development as a function of maternal body size and age; but in this case I used only *H. convergens*. Offspring were collected at three different times in female reproductive life and reared while providing unlimited food. Observations were made on developmental times and final quality and the dry masses of adult progeny obtained were recorded.

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Chapter 1 - Effects of larval food supply, and by extension maternal body size, on trajectories of reproductive allocation in *Coleomegilla maculata* and *Hippodamia convergens*

Coleomegilla maculata

Abstract

Lifetime trajectories of oviposition were followed in *Coleomegilla maculata* to determine effects of body size and age on reproductive allocation. Three sizes of beetles were produced by varying larval access to food, eggs of *Ephestia kuehniella*, and mated females were isolated with *ad libitum* food. Egg size increased over time in all treatments, the rate of increase being greatest in small females that started laying the smallest eggs, and converged on a similar size around the 20th oviposition day. Large females had greater fecundity early in life, a strategy inferred to maximize fitness on ephemeral patches of aphid prey, whereas smaller females increased their fecundity over time. The reproductive effort of large females (egg mass / body mass) remained constant over the first 30 oviposition days, but increased among small and medium females, suggesting gradual compensation for larval food deprivation. Increases in egg size with maternal age are likely adaptive for aphidophagous coccinellids as offspring produced late in an aphid cycle experience increased competition and higher risk of mortality. This study demonstrates, for the first time in Coleoptera, that dynamic changes in both egg size and number occur as a function of female age and illustrates that such changes are constrained by legacies of the larval food supply via their effects on maternal body size.

Introduction

The manner in which organisms allocate resources to reproduction is a central concept within life history theory (Sinervo 1990, Stearns 1989, Wilkinson & Gibbons 2005). Smith & Fretwell (1974) pointed out that females face a trade-off between investment of resources in propagule size versus propagule number; they may produce either a few large offspring, or many

smaller ones. Clearly, individual offspring benefit whenever egg size is favored at the expense of clutch size, but this is not always in the best interest of the parents (Stearns 1992) and the actual size and number of eggs is shaped by selection acting on maternal fitness (Roff 1992). Consequently, egg size tends to appear relatively fixed within populations, whereas clutch size tends to vary more readily in response to environmental conditions (insects, Stewart *et al.* 1991a, b; Dixon & Guo 1993; spiders, Marshall & Gittleman 1994; fish, Einum & Fleming 2000). It is often true that larger females lay more eggs than smaller ones, especially in highly fecund species such as insects (e.g., Kajita & Evans 2010). Honěk (1993) analyzed data on 68 insect species from 10 insect orders and found that fecundity scaled with female body size in a linear manner with a slope slightly less than one. In holometabolous insects, adults may vary in body size either because of genetic differences or as a result of differential access to food during larval development. Whereas female body size determines, to some extent, the 'capital' available for reproduction, temporal patterns of reproductive allocation reflect a series of tradeoffs between current versus future reproductive effort over the adult lifetime (Williams 1966). Thus, both egg size and egg number may vary as functions of maternal body size and age (Fox & Czesak 2000).

The idea that species tend to evolve towards a fixed (optimal) propagule size has been challenged by observations of taxa where offspring size may vary as much as clutch size (Stearns 1992, Roff 1992). Similarly, Bernardo (1996) argued against the use of optimality theory in analyzing the evolution of propagule size and emphasized the role of maternal effects and dynamic maternal ecologies in accounting for within-species variation. Among the most pronounced maternal effects are those deriving from a mother's size and age, although other factors such as temperature (Fischer *et al.* 2004) and maternal population density can have subtle effects (Heisswolf *et al.* 2009). Egg size plasticity can yield benefits when reproductive organisms face either spatial or temporal variation in environmental conditions over time frames shorter than their adult lifespan (amphibians, Crump 1981; fish, Hutchings 1991; insects, Fox & Mousseau 1998). Under such conditions, parental fitness is maximized by producing a range of offspring sizes, either within clutches or among them (Kaplan & Cooper 1984).

In general, females will benefit by increasing fecundity at the expense of egg size when environmental conditions are favorable for offspring development (e.g., abundant resources, reduced competition), whereas individual progeny typically require a larger allocation of parental resources in order to survive poor conditions, constraining the number that can be produced

(Parichy & Kaplan 1992, Fox & Mousseau 1998). Consequently, increased egg size has been proposed as an adaptation for coping with environmental stress. For example, in the brook trout, *Salvelinus fontinalis*, food restriction results in the production of fewer, larger eggs that give rise to juveniles with higher survival rates (Hutchings 1991). Females of the seed beetle, *Stator limbatus*, lay many small eggs in the seeds of high-quality host plants and fewer, larger eggs in those of poor quality hosts, thus providing their offspring with more initial resources when they must develop on nutritionally inferior food (Fox & Mousseau 1998). In aquaculture populations of chinook salmon, *Oncorhynchus tshawytscha*, there has been rapid, unintentional selection for smaller egg size, ostensibly because of enhanced juvenile survival under the benign conditions of captivity (Heath *et al.* 2003).

There have also been many studies of changes in propagule size as a function of female age. In species with indeterminate growth, or females that continue to grow after the onset of reproduction, older mothers may be larger, possess more resources, or be more experienced in parental care, enabling them to produce offspring that are larger, of higher quality, or both, as in the painted turtle *Chrysemys picta* (Paitz *et al.* 2007). For organisms reproducing within well-defined patches of habitat, resource competition for progeny may increase predictably over time, favoring increased provisioning of eggs and reduced numbers with advancing female age, as observed in the soil mite, *Sancassania berlesei* (Benton *et al.* 2005, Plaistow *et al.* 2007). In insects, the more common pattern is for egg size to decline with female age (see Fox & Czesak 2000 for a review) and most examples to the contrary occur in insect orders that lack complete metamorphosis (e.g., McLain & Mallard 1991, Landa 1992). Reductions in egg size due to deteriorating maternal physiology i.e., senescence may not be evident until near the end of life, but earlier declines may occur if key maternal resources become limiting (Fox & Czesak 2000).

Many species of lady beetles (Coccinellidae) specialize in feeding on aphid outbreaks that present an abundance of food for a brief period; plant suitability for aphids is typically fleeting and winged forms rapidly disperse to alternative hosts. Successful exploitation of an aphid outbreak requires that eggs be laid during the ‘oviposition window’, a brief period that coincides with the exponential growth phase of the aphid population (Kindlmann & Dixon 1993). Timing permits eclosing larvae to grow in parallel with aphid colonies – too early and progeny may eliminate their food supply; too late and the aphid colony may crash before larval development is complete. If conditions for progeny deteriorate over the oviposition period, an

adaptive maternal strategy would be to increase egg size over the reproductive period, possibly at the expense of egg number. Coccinellid larvae hatching from large eggs have generally better survival under conditions of food limitation (Ng 1988) and/or faster development (Stewart *et al.* 1991a). It has been proposed that coccinellid egg size remains near a minimum determined by the size neonate larvae require to capture their first prey item (Stewart *et al.* 1991a, Dixon 2000). However, unexplained variation in egg size among coccinellid females has been observed (e.g., Dixon & Guo 1993, Honěk *et al.* 2008b, Kajita & Evans 2010), but accounting for female size and age has been neglected in studies addressing reproductive allocation in lady beetles (Dixon and Guo 1993, Dixon 2000).

The objective of this study was to explore the relationships between female body size, age-specific reproductive effort, and the dynamics of egg size and daily fecundity over reproductive life in a coccinellid beetle, *Coleomegilla maculata* (DeGeer). By varying larval access to food, adults of different sizes of *C. maculata* were produced. Females were mated, fed *ad libitum* as adults, and their reproductive activity monitored daily to determine effects of female body size on egg size, daily fecundity, and the dynamics of these reproductive parameters as a function of female age. I hypothesized that plasticity in patterns of reproductive allocation should be adaptive in the ecological context of aphidophagy in which later offspring are predicted to face more severe conditions than those produced earlier.

Materials and methods

Insect colony

A colony of *C. maculata* was established from adult beetles collected from sorghum plants in Hays, KS, USA, in July, 2009. Insects were held in a growth chamber with L16:D8 day length at a constant temperature of $24 \pm 1^\circ\text{C}$ and relative humidity between 30 and 50%. Adult females were isolated in plastic Petri dishes (5.5 cm diam.) and were fed a diet of frozen eggs of the flour moth, *Ephesia kuehniella* Zeller (Lepidoptera: Pyralidae), obtained from a commercial insectary (Beneficial Insectary Inc. Oak Run, California). Water was provided on a small cube of sponge. Food and water were refreshed every day. Eggs were collected daily from the inner surfaces of the Petri dishes by transferring the beetles to new dishes. Larvae of the first laboratory generation were reared on frozen eggs of *E. kuehniella* and offspring of the resulting adults were used in the experiment.

Experimental design

The experiment was conducted under the same physical conditions used for rearing the beetle colony. Neonate larvae ($n = 180$) were isolated in Petri dishes upon eclosion and then randomly assigned to one of three different treatments, representing three different levels of food availability, for rearing through to the adult stage. Larvae were provided access to frozen eggs of *E. kuehniella* for various periods every day: i) 30 minutes per day (90 larvae), ii) 6 hours per day (45 larvae), and iii) *ad libitum* (45 larvae). More larvae were assigned to the 30 min regime to compensate for the relatively high mortality observed in that treatment in preliminary observations.

All insects were observed daily through the experiment, and developmental time was tallied as the number of days from eclosion to emergence of the adult. Upon emergence, adults were weighed on an analytical balance and then isolated in a Petri dish with frozen *E. kuehniella* provided *ad libitum* and water on a sponge cube, both refreshed daily. Eggs of *E. kuehniella* were used as a diet during development and reproduction instead of aphids because it is a suitable food capable of sustaining normal development and supporting successful reproduction in *C. maculata* (Michaud & Jyoti 2008) and is more convenient to supply than aphids in a long term experiment. When adults were seven days old, mating pairs were established and held together for a period of one week, whereupon males were removed and females were held in isolation for the remainder of their lives. Isolation was deemed necessary because preliminary observations indicated that, during extended periods of pair confinement, female oviposition schedules could be disrupted by male harassment and eggs were sometimes cannibalized by males before they could be harvested. The pre-reproductive period of each female was calculated as the number of days from adult emergence until the onset of oviposition. Observations were made daily on the number of eggs laid (daily fecundity), the linear dimensions of the eggs, and the fraction of eggs that subsequently hatched. Using a stage micrometer under a stereo microscope (magnification $\times 50$), we measured the length and width of each egg to the nearest 0.02 mm in a sample of eggs from each daily oviposition ($n = 5$). We assumed that eggs are ellipsoidal in shape (Takakura 2004) and used these measurements to estimate egg volume using the formula for an ellipse:

$$\text{Volume} = LW^2\pi/6$$

During preliminary work, we measured a series of eggs of varying sizes ($n = 115$) and then weighed each on a microbalance. We then used linear regression to establish the relationship between egg volume and mass, which was described by the following equation:

$$\text{Mass} = 0.8126 * \text{Volume} + 0.0226, (F_{1,114} = 994.84; P < 0.0001; r^2 = 0.89)$$

Since the weighing of individual eggs was very time-consuming (due to the high sensitivity of the microbalance), we henceforth measured eggs, calculated volumes, and converted values to egg masses using this equation, since the latter were required to estimate females' reproductive effort as a proportion of their body mass (RE). Egg size data was analyzed up to 20 oviposition days because declining fertility beyond this point impeded accurate measurements.

Statistical analysis

Treatments were compared by one-way ANOVA using PROC GLM (SAS Institute, 2008) and means separated by Tukey's HSD test. Mortality rates were analyzed by Chi Square using PROC FREQ (SAS Institute, 2008). Linear regression was used to test the relationship between reproductive days and daily fecundity, and slopes were compared with a test for equality of slopes using PROC REG followed by PROC GLM (SAS Institute, 2008). Changes in dependent variables with female age were analyzed using PROC MIXED for repeated measures where subject effects were considered random and the response variable was calculated as a sum of terms for overall mean, treatment effect, subject effect, time effect, treatment*time interaction, and random error. Sphericity was tested using PROC GLM with the response equal to the sum of terms for overall mean, treatment effect, time effect, treatment*time interaction, and random error (SAS Institute, 2008).

Results

The three larval feeding regimes (30 min, 6 h, and *ad libitum* access daily) yielded females of three different body sizes (small, medium and large, respectively) and three different developmental periods (long, medium and short, respectively; Table 1-1). Mortality of larvae was 36% in the 30 min feeding regime, significantly more ($\chi^2 = 55.43, P < 0.001$) than the 11% observed in the treatment with 6 h access, which was not significantly different ($\chi^2 = 0.24, P = 0.620$) from the 9% observed in the *ad libitum* treatment.

Larger females trended towards an onset of oviposition that was 12 days earlier than that of smaller females, but the pre-reproductive period did not differ significantly among treatments because of high within-group variance. There was no effect of treatment on egg mass when female lifetime means were compared, nor was linear regression of egg mass on female mass significant when all females were pooled ($F_{1,56} = 1.79, P = 0.186$). However, small females produced fewer total eggs than did medium and large females; although they oviposited on a similar number of days, their average daily fecundity was lower. There were no differences among treatments in the percentage of eggs hatching, nor was there any indication that fertility might vary with treatment during any particular period of reproductive life. Egg fertility declined over time in a fairly uniform and linear manner in all treatments, likely due to cumulative sperm depletion, since females had access to males for only their first week of adult life (Fig. 1-1). Although medium-sized females tended to live the longest and have the greatest reproductive effort, there were no significant differences among treatments in female longevity or in lifetime reproductive effort expressed as a multiple of female fresh mass at emergence (Table 1-1).

Changes in egg mass were evident as a function of female age in all treatments; egg mass increased from the first to the 20th day of oviposition by 14, 8, and 13% for small, medium and large females, respectively. The ANOVA for repeated measures of egg mass across oviposition day revealed no significant effect of treatment ($F_{2,54} = 2.41, P = 0.099$); females in all treatments tended to increase egg mass in the course of the first 20 oviposition days ($F_{9,449} = 32.92, P < 0.001$). However, there was a significant interaction between treatment and oviposition day ($F_{18,449} = 3.43, P < 0.001$). Longitudinal comparisons of means were not justified because analysis of orthogonal components indicated non-homogeneity of variance ($\chi^2 = 109.74, P < 0.001$), rendering pair-wise comparisons dependent on sphericity (e.g. HSD) inappropriate. Therefore, we used linear regression to analyze changes in egg mass within treatments over the first 20 oviposition days. A test for equality of slopes revealed treatment effects ($F_{2,59} = 3.57, P = 0.035$; Fig. 1-2). Egg mass increased more rapidly for small females than for medium-sized females ($F_{1,39} = 8.16, P = 0.007$) with large females intermediate and not significantly different from either ($F_{1,39} = 1.17, P = 0.286$ and $F_{1,39} = 2.35, P = 0.133$, respectively).

Linear regressions of daily fecundity versus oviposition day revealed that treatment had a significant effect on the change in daily fecundity over time (test for equality of slopes: $F_{2,179} =$

39.78, $P < 0.001$; Fig. 1-3). The mean daily fecundity of large females decreased over time, yielding a regression slope that was significantly different from that of medium ($F_{1,119} = 59.70$, $P < 0.001$) and small ($F_{1,119} = 42.94$, $P < 0.001$) females that both increased daily fecundity over time. Furthermore, the mean daily fecundity of medium-sized females increased over time at a higher rate than that of small females ($F_{1,119} = 5.33$, $P = 0.022$).

When fecundity was considered in increments of 10 oviposition days, there were significant effects of female body size on fecundity (1-10 days: $F_{2,56} = 10.45$, $P < 0.001$; 11-20: $F_{2,56} = 4.29$, $P = 0.018$; 21-30: $F_{2,54} = 3.27$, $P = 0.045$; 31-49: $F_{2,50} = 5.63$, $P = 0.006$). Medium-sized females laid more eggs than small females from oviposition days 31-40 (Tukey's test, $\alpha = 0.05$). However, large females laid more eggs than medium size females during the first 10 oviposition days (Tukey's test, $\alpha = 0.05$), with no differences significant thereafter, and more eggs than small females during the first 40 oviposition days, with no differences significant thereafter. All females demonstrated a marked decline in fecundity after 80 oviposition days.

Lifetime reproductive effort (RE), calculated as total egg mass divided by female fresh mass at emergence, was highly variable and ranged from 2.66 to 28.78 multiples of female body mass. When RE was partitioned into increments of 10 oviposition days, ANOVA for repeated measures revealed that all females tended to increase their RE over time ($F_{2,102} = 14.27$, $P < 0.001$), but the magnitude of the increase varied among treatments ($F_{2,54} = 3.80$, $P = 0.028$) and without significant interaction between treatment and interval ($F_{4,102} = 1.59$, $P = 0.183$). Whereas there was no difference among treatments with respect to RE during either the first or second series of 10 oviposition days ($F_{2,54} = 2.38$, $P = 0.102$ and $F_{2,56} = 2.49$, $P = 0.092$, respectively) small females expressed significantly higher RE than did large females during the third series, with mid-sized females intermediate and not significantly different from either ($F_{2,52} = 3.90$, $P = 0.026$; Fig. 1-4). Changes in RE over the three oviposition periods did not violate the assumption of sphericity ($\chi^2 = 2.94$, $P = 0.22$) which permitted longitudinal comparisons to reveal that both small and medium-sized females increased their RE during this period, while large females did not.

There were also treatment effects on the rate of reproductive episodes per unit time (Table 1-2). Large females produced their first 20 oviposition days in a significantly shorter period than did medium females, with small females intermediate and not different from either, although after 30 oviposition days no differences among treatments remained significant.

Discussion

The largest effect of maternal body size was on the number of eggs laid per day (daily fecundity), reflecting a consistent trend across iteroparous animals in general (Roff 1992), and insects in particular (Honěk 1993). Large females adopted a ‘front-loaded’ reproductive strategy and exhibited greater fecundity early in adult life when offspring are expected to contribute most to maternal fitness, their daily fecundity gradually decreasing over time (Fig. 1-3; Table 1-2). In contrast, small and medium females gradually increased their daily fecundity over time, possibly because they required a period of adult feeding to compensate for larval food deprivation. Daily fecundity declined in small and medium females only near the end of reproductive life (data not shown). Note that small females fed for more than a week longer, on average, than did medium-sized females before initiating oviposition, although treatment means were not significantly different due to the large variation among females.

The egg size of all females increased over the course of the first few weeks of oviposition and converged on a similar mass around the 20th oviposition day. The slope of the increase varied among treatments (Fig. 1-2); small females laid the smallest eggs initially but increased egg size more rapidly than larger females. Since the fecundity of small and medium females also increased during this period (Fig. 1-3), there was no indication of an egg size-number tradeoff in temporal trajectories of reproductive effort. Furthermore, there was no significant correlation between maternal body size and mean egg mass when all females were pooled; suggesting that egg mass was more affected by maternal age than maternal body size.

Within genetically determined limits, egg size is a maternal effect (Bernardo 1996), and the consequences of variation in egg size are typically most pronounced in adverse environments (Fox & Mousseau 1998). Although *C. maculata* is a relatively polyphagous lady beetle, larvae develop primarily in highly ephemeral patches of aphid prey (Wright & Laing 1980). Aphidophagous habits have been theorized to exert strong selection on the placement of eggs and the size, number, and timing of their production (Honěk *et al.* 2008a, Kindlmann & Dixon 2010). The food supply for developing aphid predators follows a predictable, sigmoidal trajectory ultimately terminating in the dispersal of alate aphids and intense competition among later-developing larvae for the remaining prey (Kindlmann & Dixon 1993). A gradual increase in egg size after onset of oviposition would seem an adaptive maternal strategy because progeny survival will increase as a function of their size at hatching under the stress of late-cycle

conditions, as will their rate of development (Stewart *et al.* 1991a). Larger neonates are better equipped to pursue increasingly scarce prey and have lower susceptibility to cannibalism and intraguild predation, mortality risks that escalate as aphid outbreaks mature. It has been argued that coccinellid egg size is held close to a minimum determined by the ability of newly-eclosed larvae to capture their first prey item (Dixon 1958, Stewart *et al.* 1991, Dixon & Guo 1993). However, Dixon & Guo (1993) observed variation in egg size among *C. septempunctata* females that was not explained by differences in maternal body size. Similarly, Honěk *et al.* (2008b) collected egg masses of *Coccinella septempunctata* and *Propylea quatordecimpunctata* from the field and observed average egg size to “change in parallel with trophic conditions experienced before oviposition”, an effect they described as “puzzling”. These observed variations in egg size may be partially or fully explained by effects of female age.

Although female fecundity and fertility typically decline with age after an early peak (Dixon & Agarwala 2002, Michaud & Qureshi 2006), the relatively steep decline in fertility in our study (Fig. 1-1) was likely due to sperm depletion, as females had access to males for only their first week of adult life. However, selection acts most strongly on early reproductive bouts and few coccinellid females in nature are likely to achieve the longevity or lifetime fecundity of our laboratory females; these enjoyed *ad libitum* food and protection from mortality risks, and were forced to conserve energy (i.e., they did not fly, nor expend normal amounts of effort foraging or seeking oviposition sites). Consequently, the observed lifetime means of reproductive parameters more likely represent artifacts of confinement than realistic values achievable in nature.

If the medium females in our study can be considered of average size, then small and large females represent outliers that might develop under conditions of prey scarcity or abundance, respectively. It is notable that reproductive effort (RE) tended to peak in medium-sized females, even though treatment means did not separate significantly (Table 1-1), an expectation if maternal body size were subject to stabilizing selection. Under field conditions, both small and large females likely experience fitness costs related to their extreme size. In this experiment, small females experienced significant stress as larvae that resulted in high mortality, slow development, and reduced fecundity in the experiment. However, the costs of being large will not necessarily be apparent under laboratory conditions, especially with *ad libitum* adult food. Large females would incur higher energy costs during flight, possibly resulting in reduced

dispersal ability, shorter longevity, increased predation and/or parasitism risks, or other impacts on fitness not measurable in our experiments. Whereas the RE of large females did not change over the course of the first 30 oviposition days, that of small and medium females increased, possibly because the *ad libitum* adult diet afforded some compensation for the food deprivation these females experienced during larval development (Fig. 1-4). Thus, the front-loaded reproductive strategy of large females appears well suited to maximize maternal fitness on scarce patches of ephemeral prey, especially if any costs of large body size accrue over time.

These results can be fit quite well to the model developed by Parker & Begon (1986), which assumes that larval fitness is determined by three components: the absolute investment in the egg, the number of larval competitors, and the size of the egg relative to the average size of other eggs. Within their categories (see their Table 1) aphidophagous coccinellids are likely subject to the 'hierarchy effect' which favors the production of larger clutches by larger females, particularly early in the aphid cycle when sib competition is likely more important than non-sib competition. Later in the cycle, a competition intensifies among larvae, including non-sibs, and the production of larger eggs is favored. An increase in clutch size in early reproductive life and a increase in egg size in later reproductive life is precisely what we observed in *C. maculata* females over the course of the first 20 oviposition days, and it is consistent with the general concept that increasing competition selects for more investment in individual offspring to ensure their survival (Brockelman 1975). Similarly, Plaistow *et al.* (2007) reasoned that an increase in egg size with female age in soil mites served to improve the survival of later-produced offspring that are forced to compete with older siblings.

An alternative, non-adaptive, explanation to the variation on egg size observed in *C. maculata* is that physiological constraints associated with ovariole development prevent females from producing their largest eggs initially, so that 'optimum' egg size is only achieved after a period of maturation. However, an ability to vary offspring size will be adaptive when the resources available for offspring fluctuate over short time periods (Fox & Czesak 2000, Benton *et al.* 2005, 2008), and this is true for aphidophagous coccinellids. Fischer *et al.* (2011) reasoned that the more reliably mothers can estimate environmental quality for their offspring, the more plasticity in offspring size should be favored. However, the *C. maculata* females in our experiment did not increase egg size in response to deteriorating conditions as they were all provided *ad libitum* food continuously; changes in egg size appeared to be a *de facto* effect of

oviposition sequence. Thus, in *C. maculata*, egg size variation does not hinge on a maternal response to changing environmental conditions, but rather reflects a heritable program of dynamic change likely triggered at the onset of oviposition.

Figures and tables

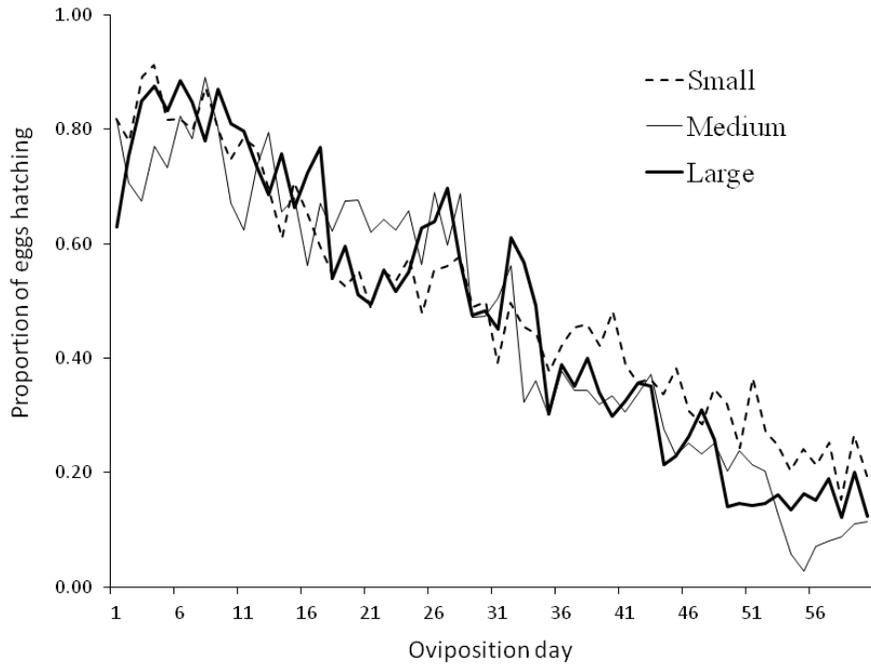


Figure 1-1 Changes in egg fertility across 60 oviposition days for females of *C. maculata* reared under three different daily larval feeding regimes: 30 min (hatched line) 6 h (thin solid line) and *ad libitum* (thick solid line) access to eggs of *E. kuehniella* that resulted in small, medium and large body sizes, respectively.

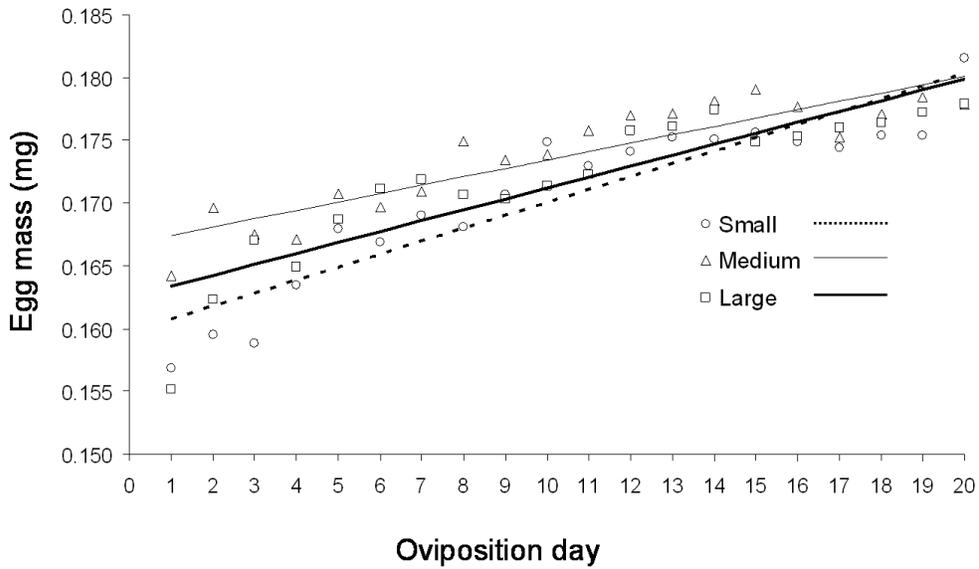


Figure 1-2 Changes in fresh mass of *C. maculata* eggs over the first 20 oviposition days laid by females reared under three different daily larval feeding regimes: 30 min (hatched line) 6 h (thin solid line) and *ad libitum* (thick solid line) access to eggs of *E. kuehniella* that resulted in small, medium and large body sizes, respectively. Regression lines: Small ($F_{19} = 101.6$, $P < 0.001$, $r^2 = 0.85$), Medium ($F_{19} = 79.8$, $P < 0.001$, $r^2 = 0.82$), and Large ($F_{19} = 65.3$, $P < 0.001$, $r^2 = 0.78$).

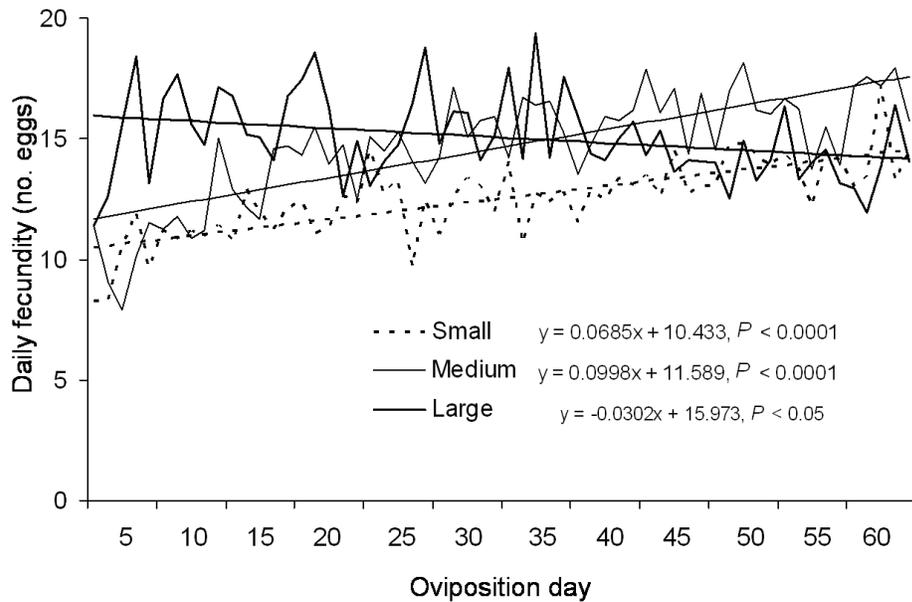


Figure 1-3 Changes in mean daily fecundity over the first 60 oviposition days by *C. maculata* females reared under three different daily larval feeding regimes (30 min, 6 h and *ad libitum* access to eggs of *E. kuehniella*) that resulted in small, medium and large body sizes, respectively. The slopes of all three regression lines were significantly different from each other in a test for equality of slopes ($\alpha < 0.05$). Regression lines: Small ($F_{58} = 73.3$, $P < 0.001$, $r^2 = 0.56$), Medium ($F_{58} = 82.7$, $P < 0.001$, $r^2 = 0.58$), and Large ($F_{19} = 5.6$, $P = 0.021$, $r^2 = 0.08$).

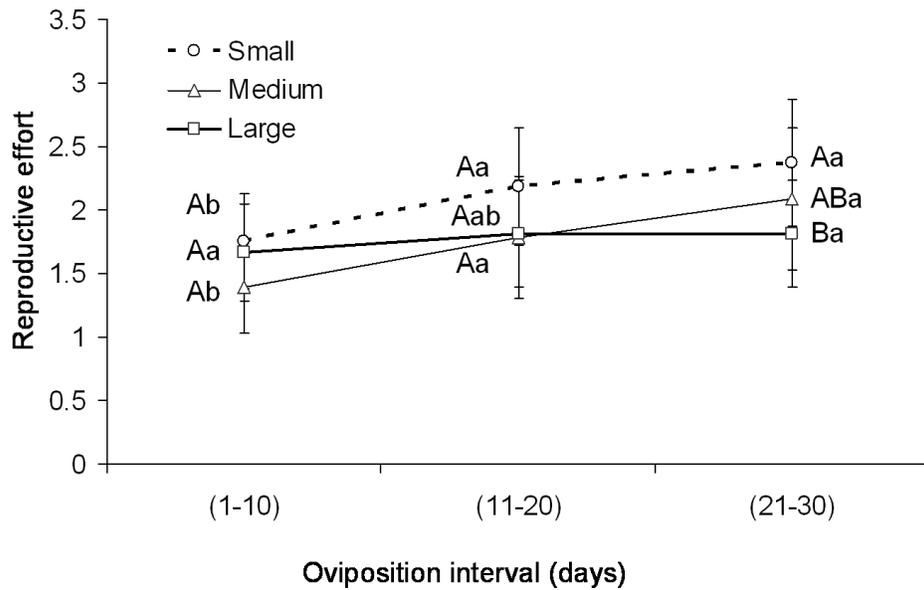


Figure 1-4 Changes in reproductive effort (total egg mass expressed as a multiple of female body mass at emergence) over the first 30 oviposition days by *C. maculata* females reared under three different daily larval feeding regimes (30 min, 6 h and *ad libitum* access to eggs of *E. kuehniella*) that resulted in small, medium and large body sizes, respectively. Values bearing the same upper case letters were not significantly different among treatments within clutch intervals; values bearing the same lower case letters were not significantly different among oviposition intervals within treatments (Tukey's HSD test, $\alpha = 0.05$).

Table 1-1 Life history parameters (mean \pm SE) of *C. maculata* females reared under three larval feeding regimes (small: 30 min, medium: 6 h, and large: *ad libitum*). As adults, each female was fed *E. kuehniella* eggs *ad libitum* and provided access to one male for the first week of adult life. Values bearing the same letters were not significantly different among larval feeding regimes (Tukey's HSD test, $\alpha = 0.05$).

	Daily larval feeding regime			df	<i>F</i>	<i>P</i>
	30 min (small)	6 h (medium)	<i>ad libitum</i> (large)			
No. females (n)	23	15	19			
Developmental time (days)	24.1 \pm 0.5a	19.6 \pm 0.2b	17.3 \pm 0.2c	57	89.4	0.0001
Adult fresh weight (mg)	9.7 \pm 0.2c	13.7 \pm 0.4b	15.6 \pm 0.3a	57	103.3	0.0001
Pre-reproductive period (days)	35.2 \pm 4.0	28.8 \pm 4.7	22.3 \pm 3.6	57	2.7	0.08
Reproductive period (d)	102.5 \pm 8.2	117.2 \pm 11.2	104.6 \pm 9.7	35	0.6	0.60
Egg mass (μ g)	172.0 \pm 2.0	176.0 \pm 3.0	174.0 \pm 2.0	56	0.8	0.50
Fecundity (total eggs)	884.4 \pm 71.6b	1,355.9 \pm 122.1a	1,263.5 \pm 134.7a	56	5.5	0.01
Total oviposition days	73.9 \pm 5.3	90.4 \pm 7.5	86.7 \pm 8.3	56	1.6	0.20
Daily fecundity (no. eggs)	12.2 \pm 0.5b	15.0 \pm 1.0a	14.9 \pm 0.8a	56	4.8	0.01
Fertility (% eggs hatching)	43.4 \pm 3.6	35.2 \pm 4.3	38.7 \pm 3.4	56	1.0	0.40
Reproductive effort*	15.9 \pm 1.3	17.7 \pm 1.4	14.3 \pm 1.5	56	1.1	0.30
Longevity (days)	137.5 \pm 6.4	142.0 \pm 10.5	129.2 \pm 9.4	35	0.5	0.60

* Total egg mass as a proportion of initial female fresh mass = (fecundity * mean egg mass) / adult mass.

Table 1-2 Mean \pm SE period of adult life (days) required to obtain 10, 20 and 30 oviposition days from *C. maculata* females of three different body sizes generated by three different daily larval feeding regimes (30 min, 6 h, and *ad libitum* access to eggs of *E. kuehniella*). Values bearing the same letters were not significantly different among oviposition days (Tukey's HSD test, $\alpha = 0.05$).

Female size	Period (d)		
	10 Ovip. days	20 Ovip. days	30 Ovip. days
Small	28.4 \pm 3.4 a	47.5 \pm 3.2 ab	61.4 \pm 3.0 a
Medium	29.9 \pm 4.3 a	50.3 \pm 4.9 a	62.3 \pm 4.7 a
Large	21.3 \pm 4.9 a	37.4 \pm 3.3 b	53.7 \pm 3.1 a
<i>F</i>	1.65	3.21	1.65
df	2,56	2,56	2,54
<i>P</i>	0.20	0.04	0.20

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Hippodamia convergens

Abstract

I explored the dynamic trajectories of offspring size and number throughout the adult lives of female lady beetles of three size classes produced by varying daily larval access to food, eggs of *Ephestia kuehniella*. Emergent females were paired with males and held with *ad libitum* food; eggs were collected daily and a subsample measured. A fraction of females in each treatment (~ 29 %) entered reproductive diapause which was broken after eight weeks by providing them with *Schizaphis graminum*. Fecundity, fertility and reproductive effort were significantly lower in diapausing females. Whereas small and medium-sized females compensated for delayed reproduction by producing larger daily reproductive bouts than their non-diapausing counterparts, large females did not, suggesting a cost of large body size for diapausing females. Among non-diapausing beetles, small females appeared reproductively constrained by larval food limitation; egg size and daily fecundity declined steadily across sequential oviposition days, as did egg fertility. Egg size in medium females increased in parallel with daily fecundity and peaked around the 18th oviposition day, whereupon both parameters declined. Large females had the largest fecundity; the increase in egg size occurred earlier than in medium females, was more pronounced, and exhibited a second period of increase once daily fecundity began to decline. Thus, there was no evidence of an egg size-number tradeoff except during the later stages of oviposition by large females. I propose that a fixed program of gradually increasing egg size, triggered at onset of oviposition, is an adaptive strategy for coccinellids that exploit ephemeral patches of aphid prey in which conditions for progeny deteriorate with predictable rapidity, favoring larger body size at eclosion and faster development.

Introduction

The concept of tradeoffs in the allocation of resources to biological processes is one that is central to life history theory (Stearns 1989, Roff 1992), whether resources are measured in terms of energy or biomass. Iteroparous organisms engage in multiple bouts of reproduction over the course of their adult lives and must allocate some proportion of their total budget of reproductive effort (RE) to each bout, or clutch, given that current investments exact a price in terms of future opportunities – the 'cost of reproduction' (Williams 1966, Bell & Koufopanou 1986). Furthermore, within each reproductive bout, females face a tradeoff between producing many small propagules, or fewer large ones (Williams 1966, Smith & Fretwell 1974, Brockelman 1975). Although egg size is usually correlated with offspring developmental time and survival, it is subject to selection acting on maternal fitness, rather than on the fitness of individual offspring (Roff 1992). Since female fitness is determined by number of offspring, one might expect females to produce eggs close to the minimum size necessary for offspring survival and maximize clutch size to the extent permitted by available resources (Einum & Fleming 2000). This reasoning leads to the general expectations that clutch size will vary more than egg size, and that larger females should produce more eggs rather than larger ones, predictions that are largely supported for highly fecund species that provide little or no parental care, as is the norm for most arthropods (Stewart *et al.* 1991, Dixon & Guo 1993, Carriere & Roff 1995, Dixon 2000, Fox & Czesak 2000). However, the minimal egg size necessary to ensure offspring survival may vary with ecological conditions, or change over the reproductive life of a female, yielding scenarios that require phenotypic plasticity in egg size to maximize maternal fitness. Thus, objections have arisen to static optimization models of egg size (Charnov & Krebs 1974, Parker & Courtney 1984) on the grounds that they do not adequately account for the critical influence of temporal and spatial environmental heterogeneity (Crump 1981, Bernardo 1996). However, Lloyd (1987) developed a model of per-offspring investment that incorporated parental care and concluded that there can be adaptive modification of offspring size in response to varying resources even if the optimal size remains under normalizing selection within the population.

When conditions for offspring become unfavorable and juvenile mortality is high, females may increase egg size if this serves to improve the likelihood of progeny survival. Such conditions include environmental stress, resource depletion, increasing competition (whether

between sibs, conspecifics, or heterospecifics), and an elevated risk of predation for small neonates. For example, acidic habitats are stressful for immature development of the moor frog, *Rana arvalis*, (Räsänen *et al.* 2008) and females lay larger eggs in low pH environments since these presumably benefit from a lower surface area-volume ratio (Räsänen, Laurila & Merila 2005). Parichy & Caplan (1992) demonstrated that small tadpoles of the frog *Bombina orientalis* took longer to develop and metamorphosed at smaller body sizes than those of larger initial size when competing with conspecifics for a limited food supply, although these disadvantages disappeared when *ad libitum* food was available. Similarly, Einum & Fleming (1999) found that differences in the growth and survival of brown trout hatching from different egg sizes were only evident in poorer growth environments with some degree of competition, leading them to conclude that optimum egg size will vary across gradients of environmental quality. Sinervo (1990) experimentally reduced egg size in the lizard *Sceloporus occidentalis* and found that the resulting smaller juveniles had lower sprint speeds which would presumably increase their risk of predation. Segers & Taborshy (2011) demonstrated that larger juveniles of the mouth-brooding cichlid *Simochromis pleurospilus* that hatched from larger eggs were not only capable of faster bursts of swimming speed, but were also able to economize on foraging effort and spend more time in predation refuges. Thus, there is evidence from multiple taxa that egg size plasticity is adaptive when offspring produced at different times or in different habitats face differential survival probabilities.

The majority of genera in the family Coccinellidae are predaceous on other insects or mites (Hodek 1996), and many are relatively specialized predators of aphids. Because of the 'boom and bust' nature of aphid populations and their ephemeral availability, aphidophagy is an ecological specialization with predictable life history consequences: rapid development, high fecundity, and a relatively fast pace of life compared to predators of prey with more diffuse distributions or more predictable availability (Dixon 2000, Borges *et al.* 2006). Furthermore, females must time their egg production to coincide with a critical stage of the aphid population cycle, the 'oviposition window', in order to maximize opportunities for the survival of their progeny (Kindlmann & Dixon 1993, 2011). Thus, females exploiting an aphid outbreak face a predictable decline in habitat quality for their offspring; prey become increasingly scarce and the intensity of competition and intraguild predation, increasingly severe, often over a time frame as short as two or three weeks.

In the present study, I tested for an effect of female body size on daily fecundity and egg size in an aphidophagous lady beetle, *Hippodamia convergens* Guerin-Meneville, and tracked trajectories of change in these parameters over the reproductive lives of individual females. In addition, since populations of *H. convergens* on the High Plains are capable of an extended period of reproductive diapause that is adaptive for surviving harsh summer conditions (Michaud & Qureshi 2006), I contrasted the partitioning of reproductive effort by females that remained in diapause for two months with that of females that began oviposition shortly after mating.

Materials and methods

Insect colony

A colony of *H. convergens* was established from adult beetles collected from cultivated sunflower, *Helianthus annuus* L., in Hays, KS, USA, in June, 2009. Insects were held in a growth chamber with L16:D8 day length at a temperature of $24 \pm 1^\circ\text{C}$ and relative humidity between 30 and 50%. Adult females were isolated in plastic Petri dishes (5.5 cm diam.) and were fed a diet of frozen eggs of the flour moth, *E. kuehniella*, obtained from a commercial insectary (Beneficial Insectary Inc. Oak Run, California), with water provided on a small cube of sponge, both refreshed every day. Eggs were collected daily from the inner surfaces of the Petri dishes by transferring the beetles to new dishes. Eclosing larvae were reared on frozen eggs of *E. kuehniella* in petri dishes (as above), five per dish, to produce the next generation of adults. Insects for the experiment were obtained from the second generation of adults produced in the laboratory.

Experimental design

The experiment was conducted under the same physical conditions used for rearing the beetle colony. Neonate larvae ($n = 200$) were isolated in Petri dishes upon eclosion and then randomly assigned to one of three different treatments, representing three different levels of food availability, for rearing through to the adult stage. Larvae were provided access to frozen eggs of *E. kuehniella* for various periods every day: i) 30 minutes per day (100 larvae), ii) 6 hours per day (50 larvae), and iii) *ad libitum* (50 larvae). More larvae were assigned to the 30 min regime because relatively high mortality was expected in that treatment.

All insects were observed daily throughout the experiment and developmental time was tallied as the number of days from eclosion to adult emergence. Upon emergence, adults were weighed on an analytical balance and then isolated in a Petri dish with frozen *E. kuehniella* provided *ad libitum* and water on a sponge cube, both refreshed daily. Eggs of *E. kuehniella* support both normal development and reproduction in *H. convergens*, although aphids such as greenbug, *Schizaphis graminum* (Rondani), are required to induce oviposition in females that have entered reproductive diapause (Michaud & Qureshi 2006). Eggs of *E. kuehniella* are more convenient food source than aphids to provide for extended periods and were not expected to affect relationships between egg size and number that were the focus of the study.

When adults were seven days old, mating pairs were established, checking parentage by recording the pedigree of the offspring used in the experiment to ensure that siblings were not paired together; females were kept with males over the course of the observations. The pre-reproductive period of each female was tallied as the number of days from adult emergence until the first reproductive episode. A fraction of the females in each treatment entered reproductive diapause, so after two months, those females failing to oviposit were provided with *S. graminum* reared on sorghum plants in a growth chamber at 24 °C. After seven days on the greenbug diet, all females had initiated oviposition and were returned to a diet of frozen *Ephestia* eggs for the remainder of the experiment. Data obtained from diapausing females were analyzed separately.

Observations were made daily on the number of eggs laid, the linear dimensions of the eggs, and the fraction of each clutch that subsequently hatched. For convenience, we use the term ‘daily fecundity’ to refer to the number of eggs laid in a daily bout of reproduction. Using a stage micrometer under a stereo microscope (magnification x50), we measured the length and width of each egg to the nearest 0.02 mm in a sample of eggs from each clutch ($n=5$). Assuming the eggs were ellipsoidal, egg volume was estimated using formula of the ellipsoid (Takakura 2004):

$$\text{Volume} = LW^2\pi/6$$

During preliminary work, we measured a series of eggs of varying sizes ($n = 146$) and then weighed each on a microbalance. We then used linear regression to establish the relationship between egg volume and mass, which was highly significant ($F_{1,145} = 1840.4$, $P < 0.001$, $r^2 = 0.92$) and described by the following equation:

$$\text{Mass} = 0.9171*\text{volume} + 0.0078$$

The weighing of individual eggs was time-consuming (due to the high sensitivity of the microbalance), so we henceforth measured eggs, calculated volumes, and converted values to egg mass using this equation, since the latter were required to estimate female reproductive effort (RE), i.e., the mass of eggs expressed as a fraction of female fresh mass at emergence.

Statistical analysis

Treatments were compared by one-way ANOVA using PROC GLM (SAS Institute 2008) followed by Tukey's HSD test to separate means. Mortality rates were analyzed by Chi Square using PROC FREQ (SAS Institute, 2008). Linear regression was used to test the relationship between oviposition day and egg mass, daily fecundity, fertility and RE. Since the lines of best fit were linear for all regressions of oviposition day on fertility, these were compared for equality of slopes using PROC REG (SAS Institute 2008). Time series observations such as changes in egg mass with female age and proportional reproductive effort with female age were analyzed using PROC MIXED for repeated measures and sphericity was tested using PROC GLM (SAS Institute 2008).

Results

Forty percent of larvae died in the 30 min feeding regime, significantly more than either the 6% observed in the 6 h treatment ($\chi^2 = 517.02$, $P < 0.001$) or the 12% observed in the *ad libitum* treatment ($\chi^2 = 218.18$, $P < 0.001$), the latter being not significantly different from one another ($\chi^2 = 1.70$, $P = 0.191$). Trends toward shorter developmental time and larger body size with increasing larval food supply were largely consistent whether females entered diapause or not. The three larval feeding regimes (30 min, 6 h, and *ad libitum* access daily) yielded three different body sizes in non-diapausing females (small, medium and large, respectively) and the 30 min treatment yielded the longest developmental times (Table 1-3). Among diapausing females, those in the 30 min treatment also took the longest to develop, whereas those in the *ad libitum* treatment were the fastest. Although diapausing females did not differ from non-diapausing females in longevity, egg mass or reproductive period, they laid only 20% as many eggs, their egg fertility was almost 30% lower, and their RE was reduced by 78% (Table 1-4). Whereas diapausing small and medium sized females exhibited relatively greater daily fecundity than their non-diapausing counterparts, ostensibly to compensate for the delay in onset of

oviposition, large females were unable to do so. Twenty four percent of females entered in reproductive diapause in the 30 min feeding regime, not significantly less than either the 31% observed in the 6 h treatment ($\chi^2 = 0.44$, $P = 0.505$) or the 32% observed in the *ad libitum* treatment ($\chi^2 = 0.56$, $P = 0.454$), the latter being not significantly different from one another ($\chi^2 = 0.64$, $P = 0.421$). In diapausing females, the small sample sizes and large within-treatment variances prevented analyses of temporal trends within treatments. Thus, the data and analyses reported hereafter pertain to non-diapausing females only.

Small females took longer to begin oviposition than large females, with medium-sized females intermediate. There was no effect of treatment on egg mass when female lifetime means were compared, but linear regression with all females pooled revealed a significant and positive relationship between female fresh mass and mean egg mass ($F_{1,36} = 4.96$, $P = 0.032$, $r^2 = 0.12$), and between female fresh mass and daily fecundity ($F_{1,36} = 26.05$, $P < 0.001$, $r^2 = 0.46$). There were strong trends toward increasing lifetime fecundity, greater number of oviposition days, greater daily fecundity, and increased RE with increasing female size but, with the exception of daily fecundity, lifetime means did not differ significantly because of large within-group variances (Table 1-3). Differences among treatments in mean fertility did not become significant until the 13th oviposition day, at which point significantly fewer eggs of small females were hatching in comparison to medium-sized females, the latter not being different from large females (Fig. 1-5). Linear regressions of fertility versus oviposition day revealed that treatment had a significant effect on the rate of decline in fertility over time (test for equality of slopes: $F_{2,74} = 21.86$, $P < 0.001$). The fertility of small females declined more rapidly than that of medium ($F_{1,49} = 35.51$, $P < 0.001$) or large females ($F_{1,49} = 10.32$, $P = 0.002$) and that of large females decreased more rapidly than that of medium females ($F_{1,49} = 14.40$, $P < 0.001$).

The ANOVA for repeated measures of egg mass across oviposition day revealed a significant effect of treatment ($F_{2,34} = 60.57$, $P < 0.001$), significant changes in egg mass over the course of the first 25 oviposition days ($F_{24,496} = 64.38$, $P < 0.001$), and a significant interaction between treatment and oviposition day ($F_{48,496} = 15.01$, $P < 0.001$). Longitudinal comparisons of means were not performed because analysis of orthogonal components indicated non-homogeneity of variance (oviposition days 1-15: $\chi^2 = 141.47$, $P = 0.008$), rendering inappropriate pair-wise comparisons that are dependent on sphericity. Changes in egg mass of large and medium-sized females were best described by third order polynomial regressions, whereas that

of small females was linear (Fig. 1-6). Whereas the egg size of large females peaked around the 10th oviposition day, that of medium-sized females did so around the 18th oviposition day, and that of small females showed a steady decline. The mean egg mass of large females increased by 4.2% over the course of the first 10 oviposition days, whereas that of medium-sized females increased by 4.5% over the first 17. In contrast, the mean egg mass of small females declined by 12.3% over the course of the first 25 oviposition days. Whereas eggs of small females were 5.4% smaller than those of large females in the 10th oviposition day, they were 14.3% smaller in the 24th oviposition day.

Changes in daily fecundity over time were best described by concave second order polynomials for large and medium-sized females, and by linear regression for small females, although only the regression for large females was significant due to high variation within treatments (Fig. 1-7). Small females tended to produce fewer eggs than big females, with medium-sized females intermediate. Daily fecundity peaked around the 12th oviposition day for large females and around the 15th oviposition day for medium-sized females. There was no significant effect of treatment on the number of days females required to produce a specific number of reproductive episodes (8th oviposition day: $F_{2,27} = 0.10$, $P = 0.902$; 16th oviposition day: $F_{2,22} = 0.95$, $P = 0.402$; 24th oviposition day: $F_{2,13} = 2.64$, $P = 0.116$).

I tested the hypothesis of a tradeoff between egg size and number by plotting the residuals of egg weight and clutch size regressions against female fresh weight according to Berrigan (1991). There was a negative relationship between residual egg weight and daily density, suggesting a tendency for females to lay fewer eggs when they were large, but the relationship was not significant ($F_{1,36} = 0.22$, $P = 0.64$).

Lifetime reproductive effort (RE), calculated as mass of total eggs divided by female fresh mass at emergence, was highly variable and ranged from 0.13 to 23.24 multiples of female body mass. By comparison, RE ranged from 0.02 to 4.38 in diapausing females of the same species. Trends in RE within treatments tended to mirror changes in daily fecundity but once again, the only significant regression was for large females (Fig. 1-8).

Discussion

The various larval feeding regimes employed in this study had dramatic effects on *H. convergens* development and subsequent reproduction in the adult stage. Although treatment-

wise comparisons among lifetime means were often not significant due to large within-group variance, certain trends were evident across treatments. Increasing food supply was associated with faster development, larger adult body mass, a shorter pre-oviposition period, larger average daily fecundity, more bouts of reproduction, greater lifetime fecundity, and greater RE (Table 1-3). A positive correlation between female body size and fecundity is common across a broad range of taxa (Roff 1992), and in insects specifically (Honěk 1993). However, it must be noted that *ad libitum* food was provided in the adult stage and that the fitness consequences of various body sizes might unfold differently under conditions of restricted food availability.

A fraction of females in all treatments entered reproductive diapause and, after a period of two months, were provisioned with aphids to stimulate oviposition. Reproductive diapause in *H. convergens* is an adaptation for surviving periods of low prey availability during hot, dry summer conditions on the High Plains (Michaud & Qureshi 2006). Diapause was costly for females; they laid only 20% as many eggs as non-diapausing females, had lower egg fertility, and reduced reproductive effort for their body size (Table 1-4). Small and medium-sized females tended to have greater daily fecundity than their non-diapausing counterparts, possibly in an effort to compensate for compression of their reproductive effort into a much shorter period of adult life. Interestingly, large females were unable to do this and trended toward producing fewer eggs than their non-diapausing counterparts, suggesting that large body size may be a disadvantage when reproduction is deferred until late in life.

Deeper insights into the dynamics of reproductive allocation are gained by analyzing changes in reproductive parameters over time and here we restrict our discussion to non-diapausing females. Larval food availability had significant effects on female fertility; small females exhibited a steep decline in fertility over time, whereas the fertility of large females declined gradually (Figure 1-5). In contrast, medium-sized females sustained relatively high fertility throughout the course of the first 25 oviposition days. Assuming body size is under stabilizing selection, medium-sized females are likely most representative of the population norm and the declining fertilities of small and large females could reflect costs associated with extremes of body size. Sperm depletion can be ruled out, as females were kept with males throughout the experiment. Coccinellid females may also vary the proportion of infertile eggs in each reproductive episode in response to variations in food availability so as to manipulate opportunities for sibling egg cannibalism, which is analogous to a form of parental care

(Michaud & Grant 2004, Perry & Roitberg 2005). However, in this case the adult food supply did not vary and was always in excess.

I conclude that the disparity among female size classes in fertility trends probably results from physiological constraints associated with different body sizes, and need not reflect size-specific life history adaptations.

It has been proposed that the life histories of aphidophagous lady beetles have been shaped by the patchy distribution and ephemeral availability of their prey such that they exhibit a 'faster pace of life' (shorter lifespan, higher reproductive rate) in comparison to coccidophagous species (Dixon 2000, Borges *et al.* 2006). Not only must females reproduce quickly when prey is available, but the optimal time frame for oviposition is short (Kindlmann & Dixon 1993) and conditions for larvae deteriorate quickly. Thus, we may expect earlier oviposition episodes to be under stronger selection than later ones as they are likely to contribute much more to maternal fitness. One result of this effect is the 'triangular fecundity function' typical of aphidopagous coccinellids (Kindlmann *et al.* 2001, Dixon & Agarwala 2002, Michaud & Qureshi 2006). However, females of different sizes exhibited different trajectories of change in both egg mass and daily fecundity (Figures 1-6 & 1-7). Large females consistently produced larger eggs than did small females, suggesting that excess resources are not simply channeled into the production of additional eggs as has been assumed (Stewart *et al.* 1991, Dixon & Guo 1993, Dixon 2000). Furthermore, the steeper increases in both egg size and daily fecundity compared to medium females suggest that large females used their greater larval legacy of resources to shift more of their reproductive effort (RE) earlier in life, a strategy of 'front-loaded' RE that would serve to maximize maternal fitness on an ephemeral resource that is rapidly exploited.

Small females appeared physiologically constrained and displayed a linear decline in both egg size and fecundity over time, suggesting that the *ad libitum* adult food supply did not afford much compensation at high levels of larval food deprivation. The monotonic decline in RE of small females from an initial maximum would seem an adaptive dynamic in light of their rapidly diminishing fertility. Changes in the egg size of medium-sized females largely paralleled changes in daily fecundity, increasing to a maximum around the 18th oviposition day, a little later than the peak in daily fecundity, and decreasing thereafter somewhat more steeply than the decline in daily fecundity. Large females increased egg size in parallel with fecundity over the first 8-10th oviposition days, but began to reduce egg size well before daily fecundity peaked

around the 15th oviposition day, suggesting they began to maintain daily fecundity at the expense of egg size during this interval. However, this trend was not sustained beyond the 20th oviposition day when egg size began to increase again as daily fecundity began a steep decline. Thus, most changes in egg size were positively correlated with changes in daily fecundity, and a tradeoff between egg number and size only became apparent late in the reproductive life of large females. Large females produced both larger eggs and more of them, especially early in their reproductive cycle; they used their surfeit of resources to produce a larger burst of reproductive effort early in life, consistent with the expectation of adaptation to an aphidophagous lifestyle.

I hypothesize that both physiological constraints and selection acting on life history interact to produce the observed trajectories of reproductive effort in *H. convergens* females. The ability of females to vary offspring size will be adaptive when the resources available for offspring fluctuate over time frames shorter than the duration of female reproductive period (Fox & Czesak 2000, Benton *et al.* 2005; 2008). The 'hierarchy effect' posited by Parker & Begon (1986) suggests that the production of large reproductive bouts of small eggs will be favored when competition for resources is low, such as when an aphid outbreak is initially colonized. However, physiological constraints associated with ovariole maturation may prevent females from maximizing daily fecundity at the very outset of oviposition, even though this might maximize maternal fitness. Similarly, physiological constraints associated with senescence likely mediate eventual declines in all components of RE late in adult life and adaptive interpretations are likely unjustified for these dynamics, especially considering that few females in nature will experience the longevities obtained under the optimal conditions that were provided in the laboratory.

Even small differences in offspring size can have important life history ramifications (Stearns 1992). Although an increase in egg size with maternal age is common in Crustacea i.e., *Daphnia magna* (Stearns 1992), it is unusual in holometabolous insects (Fox & Czesak 2000, Giron & Casas 2003). Egg size variation in insects tends to be associated with variable environmental quality (Fox & Mousseau 1998); individuals hatching from larger eggs tend to have higher survival, faster development, and often reach a larger adult size. As aphids become scarce for coccinellid larvae and the pressures of competition and intraguild predation intensify, an increase in egg size over successive reproductive episodes should be favored (Parker & Begon 1986). In analogous studies with soil mites, Plaistow *et al.* (2007) demonstrated increased

progeny size as a function of female age that they construed to be adaptive in the context of the predictable increase in competition among offspring produced later in the reproductive cycle. Changes in reproductive allocation by *H. convergens* females were not triggered by environmental deterioration, as adult food was provided *ad libitum*; rather they appeared to be a direct function of oviposition sequence/maternal age. Fischer *et al.* (2011) concluded that the more reliably females can predict environmental conditions for offspring, the more plasticity in offspring size should be favored. Since aphidophagy entails exploitation of a resource that deteriorates with predictable rapidity for successive reproductive episodes, there are adaptive reasons why *H. convergens* females should strive to increase both egg size and number early in reproductive life, to the extent their accumulated resources permit.

Our research results appear to be the first evidence of increasing egg size as a function of female age in the Coleoptera (see Fox & Czesak 2000), and the life history consequences of such dynamics have not yet been adequately explored. Females in our experiment experienced many artifacts of laboratory confinement that likely extended their reproductive periods, including protection from mortality, *ad libitum* food provision, and forced conservation of energy that would normally be expended in dispersal and foraging. Future studies could manipulate factors such as energy expenditure in non-reproductive activities and adult food limitation to determine their respective impacts on trajectories of reproductive allocation. For example, oosorption has recently been demonstrated in coccinellids (Osawa 2005, Kajita & Evans 2009) and is presumably a tactic for conserving reproductive effort for future opportunities when conditions deteriorate. The question arises whether or not egg size will 'reset to small' following resumption of oviposition in a subsequently encountered aphid patch. Egg size is a maternal effect (Fox & Mousseau 1998) and its life history consequences for offspring have not been adequately explored in these insects. Other than egg size, other, more cryptic, effects of maternal age on offspring life histories might be detectable with appropriate experimental designs. Additional studies of reproductive allocation schedules in insects and their life history consequences are warranted to further resolve dynamics reflecting adaptive phenotypic plasticity from those arising from physiological constraints.

Figures and tables

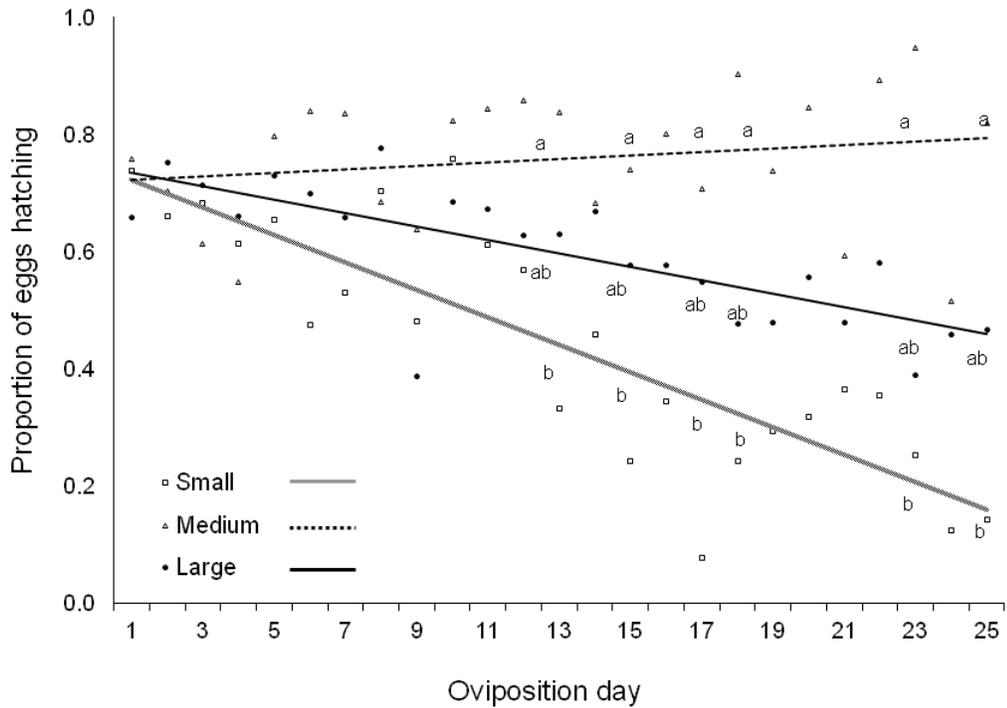


Figure 1-5 Trend lines of best fit in mean fertility (proportion of eggs hatching) over successive oviposition days by *H. convergens* females of three different size classes produced by varying larval access to food (eggs of *E. kuehniella*), and that were provided with unlimited access to food (eggs of *E. kuehniella*) and males during reproduction . (Small: $F_{24} = 56.9$, $P < 0.001$, $r^2 = 0.71$; Medium: $F_{24} = 0.9$, $P = 0.35$, $r^2 = 0.04$; Large: $F_{24} = 30.1$, $P < 0.001$, $r^2 = 0.57$). Line slopes were significantly different in all pairwise comparisons ($P < 0.001$ in all cases). Letters denote oviposition days for which differences among treatments were significant (Tukey's HSD test, $\alpha = 0.05$).

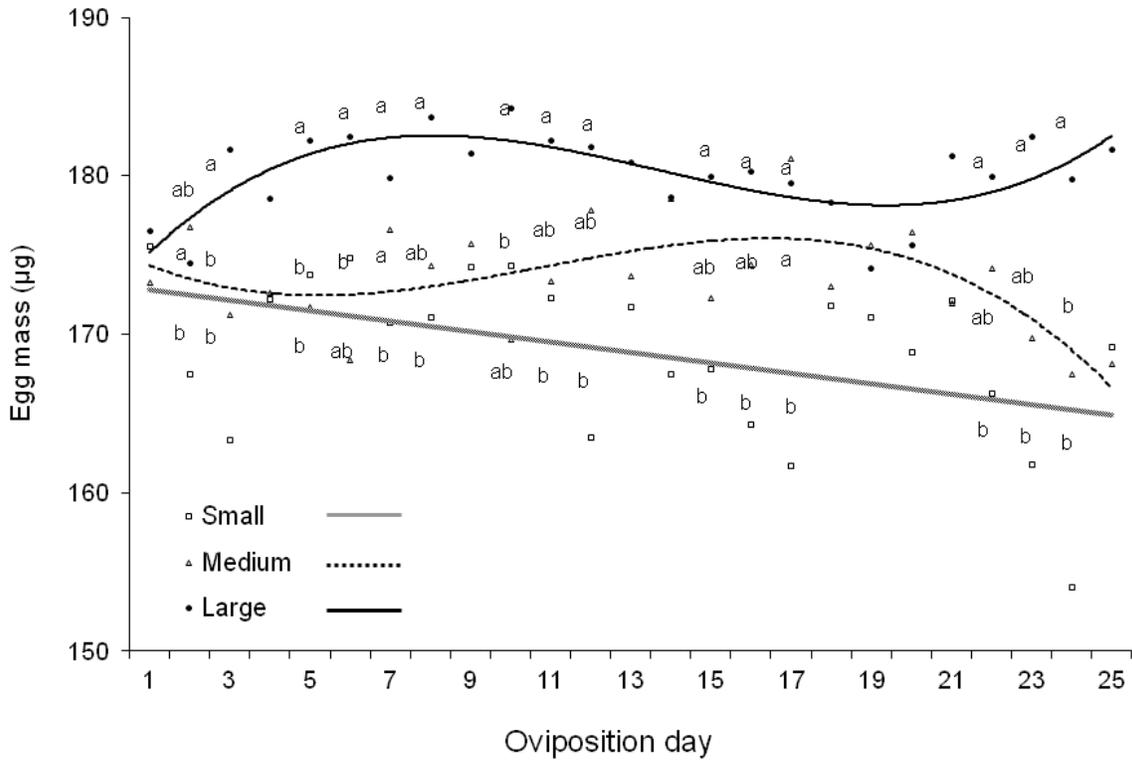


Figure 1-6 Trend lines of best fit in mean egg mass over successive oviposition days by *H. convergens* females of three different size classes produced by varying larval access to food (eggs of *E. kuehniella*) and that were provided with unlimited access to food (eggs of *E. kuehniella*) and males during reproduction. (Small: $F_{24} = 6.4$, $P = 0.01$, $r^2 = 0.21$; Medium: $F_{3,24} = 5.3$, $P = 0.007$, $r^2 = 0.42$; Large: $F_{3,24} = 7.8$, $P = 0.001$, $r^2 = 0.52$). Letters denote oviposition days for which differences among treatments were significant (Tukey's HSD test, $\alpha = 0.05$).

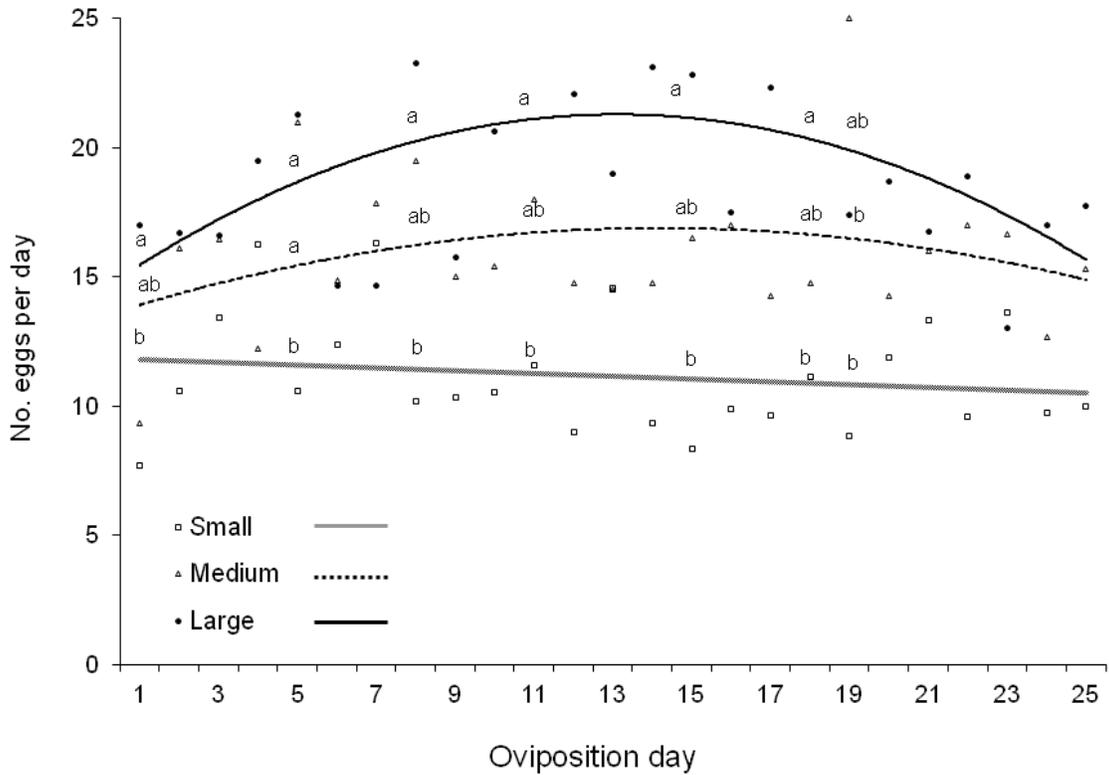


Figure 1-7 Trend lines of best fit in mean daily fecundity (no. eggs per reproductive day) over successive oviposition days by *H. convergens* females of three different size classes produced by varying larval access to food (eggs of *E. kuehniella*) and that were provided with unlimited access to food (eggs of *E. kuehniella*) and males during reproduction. (Small: $F_{2,24} = 0.7$, $P = 0.39$, $r^2 = 0.03$; Medium: $F_{2,24} = 1.0$, $P = 0.38$, $r^2 = 0.08$; Large: $F_{2,24} = 4.0$, $P = 0.03$, $r^2 = 0.27$). Letters denote oviposition days for which differences among treatments were significant (Tukey's HSD test, $\alpha = 0.05$).

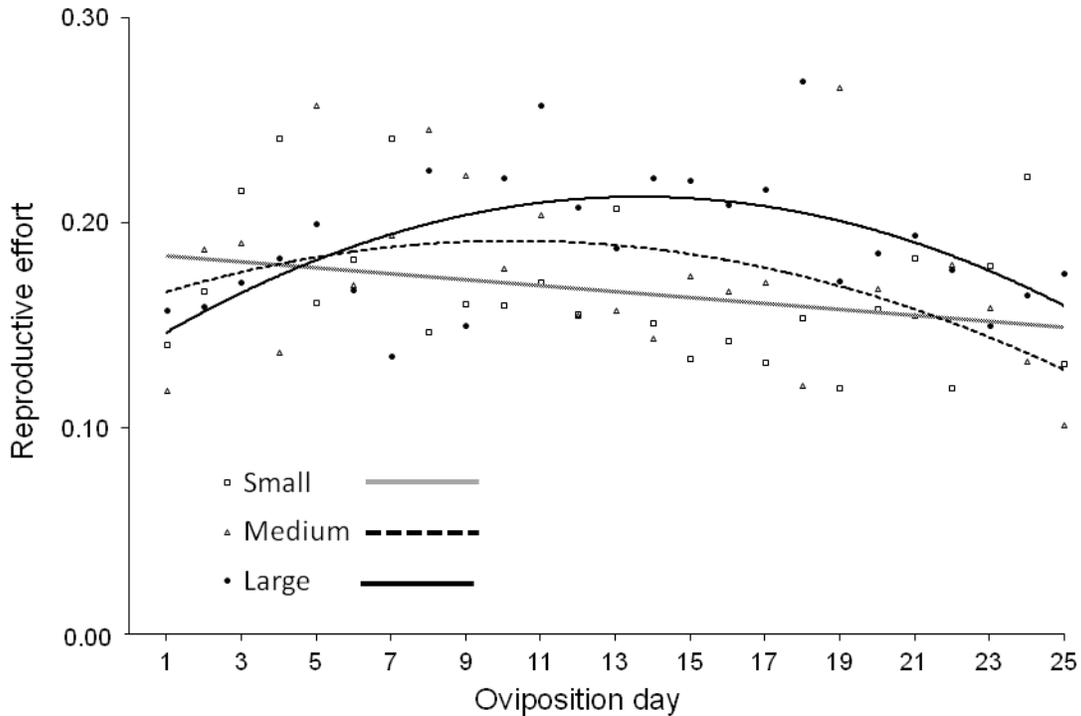


Figure 1-8 Trend lines of best fit in mean reproductive effort (RE = fresh mass of eggs / body mass of female at emergence) over successive oviposition days by *H. convergens* females of three different size classes produced by varying larval access to food (eggs of *E. kuehniella*) and that were provided with unlimited access to food (eggs of *E. kuehniella*) and males during reproduction. (Small: $F_{2,24} = 2.4$, $P = 0.13$, $r^2 = 0.09$; Medium: $F_{2,24} = 2.6$, $P = 0.09$, $r^2 = 0.18$; Large: $F_{2,24} = 6.0$, $P = 0.008$, $r^2 = 0.35$). Letters denote oviposition days for which differences among treatments were significant (Tukey's HSD test, $\alpha = 0.05$).

Table 1-3 Life history data (mean \pm SE) for *H. convergens* females reared under three feeding regimes (small: 30 min, medium: 6 h, and large: *ad libitum*). As adults, each female was fed *E. kuehniella* eggs *ad libitum* and held with a male for the duration of their reproductive life. Females that entered reproductive diapause were fed *Schizaphis graminum* at \sim 8 weeks post-emergence to induce oviposition, began laying eggs 4.4 ± 0.5 d later, and were switched to *E. kuehniella* eggs after 7 d on aphids. Means bearing the same letter were not significantly different within rows (Tukey's test, $\alpha = 0.05$).

Parameter	Daily larval feeding regime			df	F	P
	30 min (small)	6 h (medium)	<i>ad libitum</i> (large)			
Non-diapausing females						
Total no. females	13	11	13			
Developmental time (d)	29.8 \pm 1.4a	22.4 \pm 0.3b	19.4 \pm 0.2b	36	36.3	0.001
Adult fresh mass (mg)	11.0 \pm 0.7c	15.2 \pm 0.3b	18.7 \pm 0.6a	36	42.8	0.001
Pre-oviposition period (d)	54.1 \pm 3.9a	42.8 \pm 4.0ab	38.3 \pm 3.5b	36	4.9	0.01
Reproductive period (d)	91.0 \pm 17.9	80.1 \pm 21.1	104.8 \pm 18.7	26	0.4	0.66
Egg mass (μ g)	168.0 \pm 2.2	174.0 \pm 5.7	178.0 \pm 3.8	36	1.7	0.19
Fecundity (total eggs)	221.8 \pm 49.4	415.1 \pm 169.7	645.3 \pm 156.8	36	2.7	0.08
Oviposition days	19.8 \pm 4.3	26.0 \pm 9.9	35.7 \pm 7.7	36	1.2	0.30
Daily fecundity	10.3 \pm 0.8b	12.9 \pm 1.2b	17.1 \pm 1.4a	36	8.6	0.001
Fertility (% eggs hatching)	51.0 \pm 5.8	61.0 \pm 6.0	53.0 \pm 5.8	36	0.7	0.49
Reproductive effort*	3.1 \pm 0.6	4.5 \pm 1.8	6.3 \pm 1.7	36	1.3	0.29
Longevity (d)	143.5 \pm 16.6	125.1 \pm 19.2	142.2 \pm 19.7	26	0.3	0.75
Diapausing females						
Total no. females	4	5	6			
Developmental time (d)	29.0 \pm 2.4a	22.6 \pm 0.2b	19.6 \pm 0.6b	14	15.3	0.001
Adult fresh mass (mg)	13.7 \pm 1.5b	15.0 \pm 0.6b	19.8 \pm 0.2a	14	17.3	0.001
Pre-oviposition period (d)	60.0 \pm 2.7b	66.0 \pm 0.3a	68.6 \pm 1.0a	14	8.9	0.004
Reproductive period (d)	50.0 \pm 15.0	104.5 \pm 17.4	33.7 \pm 11.0	8	5.9	0.03
Egg mass (μ g)	164.0 \pm 7.0	166.0 \pm 5.0	170.0 \pm 7.0	14	0.2	0.78
Fecundity (total eggs)	78.0 \pm 36.2	143.0 \pm 60.8	46.3 \pm 18.1	14	1.6	0.24
Oviposition days	6.0 \pm 3.1	8.6 \pm 3.2	4.5 \pm 1.8	14	0.6	0.53
Daily fecundity	12.5 \pm 4.4	17.9 \pm 2.7	10.7 \pm 3.5	14	1.2	0.34
Fertility (% eggs hatching)	39.0 \pm 16.0	34.0 \pm 8.0	42.0 \pm 17.0	14	0.1	0.91
Reproductive effort*	1.1 \pm 0.5	1.6 \pm 0.7	0.4 \pm 0.1	14	1.8	0.20
Longevity (d)	138.0 \pm 14.9	193.2 \pm 17.5	122.2 \pm 10.0	8	6.1	0.03

* Total egg mass as a proportion of initial female fresh mass = (fecundity * mean egg mass) / adult mass.

Table 1-4 Life history data (mean \pm SE) for *H. convergens* females that entered in reproductive diapause versus those that did not (each group was pooled from all three feeding regimes). As adults, each female was fed *E. kuehniella* eggs *ad libitum* and provided access to one male during whole life. Females that entered reproductive diapause were fed *Schizaphis graminum* at \sim 8 weeks post-emergence to induce oviposition, began laying eggs 4.4 ± 0.5 d later, and were switched to *E. kuehniella* eggs after 7 d on aphids. Means bearing the same letter were not significantly different within rows (Tukey's test, $\alpha = 0.05$).

	Female group		df	F	P
	Non-diapausing	diapausing			
Reproductive period (d)	92.9 \pm 10.8	68.8 \pm 14.1	35	1.4	0.24
Egg mass (μ g)	173.7 \pm 2.5	167.4 \pm 3.9	51	1.9	0.17
Fecundity (total eggs)	428.1 \pm 80.1a	87.1 \pm 24.4b	51	7.1	0.01
Oviposition days	27.3 \pm 4.3a	6.3 \pm 1.5b	51	9.3	0.01
Daily fecundity (Small and Medium females)	11.5 \pm 0.7b	15.5 \pm 3.5a	32	4.2	0.04
Daily fecundity (Large females)	17.1 \pm 1.4	10.7 \pm 0.7	32	4.1	0.05
Fertility (% eggs hatching)	54.9 \pm 3.5a	39.0 \pm 7.2b	51	4.9	0.03
Proportional reproductive effort*	4.6 \pm 0.8a	1.0 \pm 0.3b	51	7.7	0.01
Longevity (d)	137.6 \pm 10.5	157.2 \pm 14.1	35	1.0	0.33

* Total egg mass as a proportion of initial female fresh mass = (fecundity * mean egg mass) / adult mass.

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Chapter 2 - Interaction of larval food supply (capital) with resource availability during the first two months of reproduction (income) in *Coleomegilla maculata* and *Hippodamia convergens*

Abstract

Lady beetles such as *Coleomegilla maculata* and *Hippodamia convergens* are primarily ‘income’ breeders in that female reproductive effort is largely dependent on resources available during reproduction. However, there is also an effect of ‘capital’, the amount of food acquired during the larval stage, that is reflected in adult body size via size-related effects on reproduction. Three size classes of beetles were produced by providing different levels of food availability during development and then mated females were subjected to alternating periods of food surplus and deficit for the first two months of their reproductive lives during which observations were made on the size and number of offspring. Whereas both species exhibited variation in daily fecundity that reflected fluctuations in adult food supply, egg size followed a fixed, species-specific pattern of change that was a function both of female age (oviposition sequence) and maternal body size. Increasing egg size as a function of advancing reproductive age would be adaptive if later-produced offspring face increasingly difficult environmental conditions such that size at eclosion becomes more strongly correlated with offspring survival. Greater female investment in egg size late in the oviposition cycle is consistent with adaptation to an aphidophagous lifestyle in which offspring are predicted to face increasing competition for food and higher risks of cannibalism and intraguild predation as aphid outbreaks cease and prey availability declines. Fertility was largely unaffected by fluctuations in adult food supply but appeared to be constrained by female body size, especially in *H. convergens*. Dynamic changes in reproductive effort (daily mass of eggs / female mass at emergence) mirrored those observed in daily fecundity, the amplitude of fluctuations being much greater in *H. convergens*, the species with higher reproductive effort. The observed dynamics of reproductive allocation would be consistent with a higher degree of aphid-specificity in *H. convergens*, since exploitation of ephemeral aphid resources is expected to select for quick adult maturation and rapid reproductive responses to take advantage of available but short-lived food supplies.

Introduction

Plants and animals have been categorized as either capital or income breeders according to whether they reproduce using resources previously stored or those obtained during the reproductive period (Stearns 1992, Jönsson 1997). Among capital breeders, body size tends to be a primary determinant of maternal fitness since it is correlated with energy stores (Morse & Stephens 1996, Colasardo *et al.* 2009) and may play a role in sexual dimorphism with females significantly larger than males (Javois *et al.* 2011). Since capital breeding effectively uncouples reproduction from foraging in both time and space, it allows animals to adjust the timing of reproductive bouts to maximize offspring fitness independent of current resource availability. However, it also implies various physiological and behavioral costs. Costs include metabolic costs associated with the conversion, deposition, maintenance and ultimate mobilization of nutrient reserves, increased costs of locomotion due to greater body mass, reduced agility (with attendant consequences for foraging efficiency and predator avoidance) and the possibility of discrepancies between nutrient reserves and reproductive demands when the resource pool is fixed prior to breeding (Jönsson 1997).

Holometabolous insects are a useful group for examining the ecology of capital versus income breeding because complete metamorphosis creates a modular life history that partitions the functions of growth and reproduction into discrete life stages, larva and adult. Since growth is determinate, adult size, and thus capital, is fixed prior to maturity. The order Lepidoptera contains a number of families in which adults do not feed after metamorphosis, rendering them obligate capital breeders (Tammaru & Haukioja 1996). In the extreme, capital breeding results in semelparity, but it is doubtful that any insects can be considered exclusively income breeders given the legacies of larval diet on the adult phenotype, especially its effect on body size. Even among adult-feeding species, certain proteins, lipids or other nutrients critical for reproduction may be acquired primarily during the larval stage (Boggs 1997, O'Brien *et al.* 2002, Casas *et al.* 2005, McCann *et al.* 2009). When both life stages consume proteinaceous food, larval and adult diets may still interact in ways that influence reproductive success (Michaud & Jyoti 2007).

Income-breeding Lepidoptera and Hymenoptera typically display considerable disparity in diet between life stages, the larvae developing as herbivores or parasites of plants or other insects and the adults often consuming only nectar or other carbohydrate-rich food sources. In contrast, there is a similarity between larval and adult diets in aphidophagous Coccinellidae and

a shared diet diminishes the likelihood that some nutrients may be accumulated as capital and others obtained as income (Boggs 1997, Casas *et al.* 2005). Although both adults and larvae may supplement their diet with vegetable matter (e.g., pollen and nectar), aphids are typically the primary source of protein and lipids for both larval growth and adult reproduction.

Aphidophagous coccinellids are primarily income breeders (Agarwala & Dixon 1992) given that adult females do not emerge with mature eggs and therefore must consume suitable prey before vitellogenesis can occur (Ferran *et al.* 1984, Stewart *et al.* 1991, Honek 1996, Michaud & Qureshi 2006). However, because of the ephemeral nature of aphid blooms, and the fact that the generation time of coccinellids is substantially longer than that of their aphid prey (Dixon 2000), most individuals do not reproduce within the same patch of aphids that supported their development. On the High Plains of the U.S.A., adult lady beetles that mature on cereal aphids in spring then normally survive a hot, dry summer in reproductive diapause to reproduce in the fall, whereas those maturing in fall hibernate through winter before reproducing in spring. Asynchrony between larval and adult food supplies is predicted to favor the use of capital resources for breeding (Chapin *et al.* 1990, Jönsson 1997). In addition, fecundity tends to scale with body size among coccinellid species (Kajita & Evans 2010), and females of most species tend to be larger and more voracious than males (Lucas *et al.* 1997; Farhadi *et al.* 2010), both characters are associated with capital breeding. In Chapter 1, the effects of maternal body size on reproductive allocation in both *C. maculata* and *H. convergens* were explored and it was demonstrated that nutritional capital has both quantitative and qualitative influences on female reproductive potential in these species when adult food supply is unlimited. Thus, the goal of this study was to examine the consequences of adult food limitation on reproduction.

In the Coccinellidae, fluctuations in the adult food supply tend to result in changes in clutch size (i.e, fecundity), rather than egg size, which some authors have assumed is held close to a minimum value determined by the critical size that neonates require to capture their first prey item (Dixon & Guo 1993, Dixon 2000). Whereas various studies have addressed the role of income in lady beetle reproduction, none have yet addressed how capital might influence income deployment (i.e., if changes in egg size versus egg number occur as a function of maternal body size). Variation in egg size has been reported in *Coccinella septempunctata* L. and *Propylea quatuordecimpunctata* L. (Honěk *et al.* 2008), although the underlying mechanisms have remained largely unexplained. In the first chapter it was demonstrated that variation in egg size

and number are a function of both female body size and timing of oviposition (early vs. late) in both *H. convergens* and *C. maculata*. These changes, in particular the tendency for egg size to increase with female age, were construed to be adaptive because offspring produced later in the aphid population cycle face a deteriorating food supply and an increasingly competitive environment, conditions inferred to favor larger propagule size (Sibly & Calow 1984, Bernardo 1986).

The objective of the present study was to measure the relative importance of nutritional capital to coccinellid reproductive allocation under conditions of income fluctuation (a surplus adult food supply interrupted by periods of food deprivation). Females of three different size classes were produced in each of two coccinellid species by limiting their daily access to food during larval development. Once females began oviposition, observations were made on their reproductive responses to alternate cycles of prey surplus/deficit produced by varying daily access to food. I hypothesized large females with greater capital reserves would exhibit greater fecundity, lay larger eggs, and be more resilient to income fluctuations than small females. If changes in egg size are an adaptation for deteriorating conditions for offspring laid later in the female reproductive life, the second hypothesis was that daily fecundity would change in response to fluctuations in maternal resources, whereas egg size would change as a function of maternal age and be independent of maternal income. Of the two species, *H. convergens* relies more heavily on aphids for reproduction than *C. maculata*. In addition, *H. convergens* is shorter-lived than *C. maculata*, and it also has a higher reproductive rate, and generally a shorter life cycle (sensu Borges *et al.* 2006). I hypothesized that the higher degree of aphid specificity in *H. convergens* would render this species more sensitive to fluctuations in the adult food supply than *C. maculata*.

Materials and methods

Insect colony

Colonies of *C. maculata* and *H. convergens* were established using adult beetles collected from sorghum infested with *Rhopalosiphum maidis* (Fitch) in Hays, Kansas, USA, in August, 2009 and June, 2010, respectively. Both colonies were maintained in a growth chamber with L16:D8 photoperiod at a constant temperature of $24 \pm 1^\circ\text{C}$ and relative humidity between 30 and 50%. For oviposition, females were isolated in plastic Petri dishes (5.5 cm diameter) and fed

with frozen eggs of the flour moth, *Ephestia kuehniella* Zeller, obtained from a commercial insectary (Beneficial Insectary Inc. Oak Run, California). Water was provided on a small cube of sponge. Food and water were refreshed daily and eggs, mostly laid on the inner surfaces of the Petri dishes, were collected daily by transferring the beetles to new dishes. Eclosing larvae were reared on frozen eggs of *E. kuehniella* in Petri dishes (as above), five per dish, to produce a second generation of adults that were used to produce the larvae cohort for our experiments.

Experimental design

The experiment with *C. maculata* was conducted between February and May 2010, whereas that with *H. convergens* was conducted between September and December 2010. Both experiments were conducted under the same physical conditions used for rearing the beetle colonies. The cohorts of larvae were started with 30 mothers in each experiment. Neonate larvae (*C. maculata*: $n = 200$, *H. convergens*: $n = 140$) were isolated in Petri dishes and then randomly assigned to one of three different feeding treatments for rearing. Larvae were provided daily access to frozen eggs of *E. kuehniella* for either 30 minutes (*C. maculata*: $n = 100$ larvae, *H. convergens*: $n = 60$), 6 hours (*C. maculata* $n = 50$, *H. convergens* $n = 40$), or *ad libitum* (*C. maculata*: $n = 50$ larvae, *H. convergens*: $n = 40$). More larvae were assigned to the 30 min regime because relatively high mortality was expected in that treatment.

All insects were observed daily throughout the experiment and developmental time was tallied as the number of days from eclosion to adult emergence. Upon emergence, adults were weighed on an analytical balance and then isolated in Petri dishes (as above) with frozen *E. kuehniella* eggs provided *ad libitum* and water on a sponge cube, both refreshed daily. Females were mated at seven days post-emergence using a male from the same treatment group and similar emergence schedule and parentage was checked to prevent the pairing of siblings. Although eggs of *E. kuehniella* support normal development in both species and are highly suitable for *C. maculata* adult reproduction, prior work indicated that aphids (e.g., greenbug, *Schizaphis graminum* (Rondani)) are required to reliably induce oviposition in *H. convergens*, and to prevent any females from entering reproductive diapause. For this reason, adults of *H. convergens* were switched to a diet of greenbugs the day pairs were established, the aphids obtained from colonies reared on sorghum seedlings in a growth chamber under the same physical conditions as the beetles, whereas *C. maculata* remained on a diet of *E. kuehniella* eggs.

Males were shifted daily among females within each treatment group during the first two weeks of the experiment, again checking to ensure siblings were never paired, whereupon males were removed to prevent the possibility of subsequent egg cannibalism. Alternating periods of food surplus/deficit were established for each female in accordance with her emergence schedule and beginning on the day she was mated; no oviposition occurred prior to the introduction of males. Two weeks of *ad libitum* food access was followed by two weeks of 6 hours daily access, followed by a repeat of the cycle. The 6 hour daily access treatment was selected on the basis of preliminary observations which revealed that lower levels of food availability completely curtailed oviposition in both species, which would have precluded collection of egg data during food deprivation periods.

The pre-reproductive period of each female was tallied as the number of days from adult emergence until the first oviposition. Observations were made daily on the number of eggs laid, the linear dimensions of the eggs, and the fraction of each batch of eggs that subsequently hatched. For convenience, we use the term ‘daily oviposition’ to refer to the number of eggs laid in a daily bout of reproduction. Using a stage micrometer under a stereo microscope (magnification x50), we measured the length and width of each egg to the nearest 0.02 mm in a sample of eggs from each clutch ($n = 5$). Egg volume was estimated based on the assumption that eggs are ellipsoidal in shape (Takakura 2004):

$$\text{Volume} = LW^2\pi/6$$

During preliminary work, we measured a series of eggs of varying sizes obtained from each species (*H. convergens*: $n = 146$, *C. maculata*: $n = 115$ eggs) and then weighed each egg on a microbalance. We then used linear regression to establish the relationship between egg volume and mass according to the following equations:

$$H. \textit{convergens}: \text{mass} = 0.9171 * \text{vol} + 0.0078 (F_{1,145} = 1840.39; P < 0.0001; r^2 = 0.92)$$

$$C. \textit{maculata}: \text{mass} = 0.8126 * \text{vol} + 0.0226 (F_{1,114} = 994.84; P < 0.0001; r^2 = 0.89)$$

We required egg mass to estimate female reproductive effort (RE), i.e., the mass of eggs laid by a female expressed as a fraction of her fresh mass at emergence. Since the weighing of individual eggs was time-consuming due to the high sensitivity of the microbalance, eggs obtained in the experiments were measured under the microscope, their volumes calculated, and then converted to egg weights using the equations above.

Statistical design

Treatments were compared by one-way ANOVA using PROC GLM (SAS Institute 2008) followed by Fisher's LSD test to separate means. Polynomial regression was used to describe trajectories of change in egg mass, daily fecundity, fertility and RE relative to the schedule of food availability. The polynomials of best fit were obtained by adding terms for the independent variable (days) with successively higher exponents until doing so failed to increase either the value of R^2 or the significance level, determined by the F test statistic:

$$F = df_j (r^2_j - r^2_{j-1}) / (1 - r^2_j)$$

where r^2_i is the r^2 for the i^{th} order, and r^2_j is the r^2 for the next higher order. Using the regression parameters obtained by PROC REG (SAS Institute 2008) we compared the r^2 for lower power equations versus the r^2 for the higher power equations. Lines of best fit were considered to be those that yielded the highest r^2 values, which in all cases yielded the highest F values. Note that each female was seven days old on day one of all graphs.

Results

The three larval feeding regimes yielded females of three different body sizes in both species with corresponding differences in developmental time (Table 2-1). The 30 min feeding treatment resulted in 16.6% mortality of *C. maculata* larvae, significantly more than the 7.5% observed in the 6 h treatment ($\chi^2 = 7.26$, $P = 0.007$), but not significantly different from the 10% observed in the *ad libitum* treatment ($\chi^2 = 2.96$, $P = 0.085$), with the latter two treatments not significantly different from one another ($\chi^2 = 0.27$, $P = 0.598$). Mortality of *H. convergens* larvae was 57% in the 30 min feeding regime, significantly more ($\chi^2 = 49.94$, $P < 0.001$) than the 26% observed in the 6 h treatment access, and the 24% observed in the *ad libitum* treatment ($\chi^2 = 59.70$, $P < 0.001$), with the latter two treatments not significantly different ($\chi^2 = 0.11$, $P = 0.740$).

Although treatment means were not significantly different, there was a trend for larger *C. maculata* females to begin oviposition earlier than smaller ones; in contrast, large and medium *H. convergens* females had longer preoviposition intervals than small ones (Table 2-1). There was an effect of treatment on egg weight in both species; larger females laid larger eggs than small females, with medium females intermediate. Whereas the fecundity of *H. convergens* over the course of the experiment did not differ significantly among treatments (largely because of high within-group variance), small *C. maculata* females had significantly lower fecundity than

medium and large females. There was no treatment effect on the number of oviposition days in either species, but the average daily fecundity of small females was lower than that of medium and large females in both species. There were no differences among treatments in the percentage of eggs hatching in *C. maculata*, but in *H. convergens* small females showed lower fertility than that of medium and large sized females, the latter not being significantly different from one another. Although small females of *H. convergens* had the highest RE during the experiment, the differences among treatments were not significant. In contrast, medium sized *C. maculata* females had the highest RE, but again, differences among treatment means were not significant.

Changes in daily fecundity in response to fluctuations in food availability were evident in both medium and small *C. maculata* females (Fig. 2-1A) and were best explained by a fifth order polynomial regression in both cases ($F_{5,21} = 9.16, P < 0.001, r^2 = 0.68$ and $F_{5,19} = 4.69, P = 0.005, r^2 = 0.55$, respectively). Although a third order polynomial provided the best description of the data for large *C. maculata* females, it was only marginally significant ($F_{3,25} = 2.77, P = 0.066$). In *H. convergens* (Fig. 2-1B), fifth order polynomials best described daily fecundity fluctuations in small, medium and large females and regressions were significant in all cases ($F_{5,22} = 9.32, P < 0.001, r^2 = 0.68$; $F_{5,22} = 13.03, P < 0.001, r^2 = 0.74$; and $F_{5,22} = 8.00, P < 0.001, r^2 = 0.64$, respectively). Periods of food surplus were associated with increases in egg number in all treatments, whereas periods of food deficit were associated with decreases in egg number across all female body sizes.

There was no indication that *C. maculata* egg mass changed in response to changes in adult food availability in any treatment (Fig. 2-2A), but regression revealed a positive linear correlation between egg mass and oviposition day (female age) in medium and small females ($F_{1,26} = 24.05, P < 0.001, r^2 = 0.49$ and $F_{1,23} = 8.55, P = 0.007, r^2 = 0.28$, respectively), whereas that of large females was best described by a negative quadratic function that peaked around day 33 during a period of food deficit ($F_{2,25} = 7.83, P = 0.002, r^2 = 0.40$). Here, egg mass increased to a peak at about 30 days, after which it gradually decreased (Fig. 2-2A). Although a sixth order polynomial best described changes in egg mass for large females of *H. convergens* females (Fig. 2-2B), it was still not significant ($F_{6,20} = 0.87, P = 0.533, r^2 = 0.20$). Whereas changes in the egg mass of medium *H. convergens* females were significantly described by a sixth order polynomial ($F_{6,20} = 5.47, P = 0.001, r^2 = 0.62$). In general, changes in egg mass for large and medium females showed increases in egg mass during periods of food surplus, whereas a decrease in egg

mass occurred in periods of food deficit. Changes in egg mass for small females were best described by a negative quadratic polynomial ($F_{2,25} = 15.27$, $P < 0.001$, $r^2 = 0.55$), and seemed unrelated to fluctuations in food availability.

Changes in daily fertility (percent eggs hatching) of large *C. maculata* females were best described by a cubic polynomial ($F_{3,25} = 0.79$, $P = 0.511$), whereas those for medium and small females were best described by negative quadratic ($F_{2,26} = 40.41$, $P < 0.001$, $r^2 = 0.77$) and cubic ($F_{3,24} = 4.19$, $P = 0.017$, $r^2 = 0.37$) functions, respectively (Fig. 2-3A). These changes did not appear to reflect fluctuations in the food supply, with the possible exception of a slight increase in the fertility of small and large females as a delayed response to the second period of food surplus. The fertility of *H. convergens* females declined monotonically over time, although at different rates in each female size class (Fig. 2-3B). Negative linear regressions were significant for small ($F_{1,25} = 114.75$, $P < 0.001$, $r^2 = 0.82$) and large ($F_{1,26} = 34.35$, $P < 0.001$, $r^2 = 0.57$) females, but not quite significant for medium females ($F_{1,25} = 3.82$, $P = 0.061$, $r^2 = 0.13$). The fertility of small females declined more rapidly than did that of large females (test for equality of slopes: $F_{1,51} = 17.7$, $P < 0.001$).

Fifth order polynomials best described changes in *C. maculata* daily RE (Fig. 2-4A) for both small ($F_{5,18} = 2.77$, $P = 0.049$, $r^2 = 0.43$) and medium ($F_{5,21} = 12.40$, $P < 0.001$, $r^2 = 0.74$) females, whereas those for large females were best described by a cubic function ($F_{3,25} = 4.31$, $P = 0.015$, $r^2 = 0.37$). Whereas the two peaks of RE occurred during periods of food surplus in small and medium females, with valleys occurring during periods of food deficit, the first period of food deficit appeared to have no effect on the RE of large females, although a decline did correspond with the second. The daily changes in RE of *H. convergens* females (Fig. 2-4B) were consistently explained by significant fifth order polynomials in females of all sizes (small: $F_{5,22} = 8.11$, $P < 0.001$, $R^2 = 0.65$; medium females: $F_{5,22} = 11.7$, $P < 0.001$, $r^2 = 0.72$; large: $F_{5,22} = 6.78$, $P < 0.001$, $r^2 = 0.60$) and mirrored fluctuations in food availability quite consistently with peaks and valleys corresponding to periods of surplus and deficit, respectively.

Discussion

Larval feeding treatments affected female body size at emergence and, in turn, the size and number of offspring produced during periods of both food surplus and food deficit. On average, small females of both species produced fewer and smaller eggs than larger females

(Table 2-1) and their RE varied more in response to changes in food availability (Fig. 2-4), supporting the first hypothesis that the greater capital of large females would afford them reproductive advantages when income varied. Thus, these results support the contention of Jönsson (1997) that capital may increase the total resources available for breeding under conditions of food limitation, even in animals that rely mainly on income to fund reproductive effort. Most of the temporal variation in RE (Fig. 2-4) appeared to take the form of variation in daily fecundity (Fig. 2-1), in accordance with the second hypothesis, and to the expectation that reproductive effort is almost directly proportional to the number of eggs being produced (Stearns 1992), but changes in egg size were inconsistent between species.

Although the egg mass of *C. maculata* females tended to increase over time and appeared insensitive to income levels as hypothesized (Fig. 2-2), fluctuations were evident in the egg mass of medium and large *H. convergens* females that appeared to coincide with changes in the maternal food supply. On the other hand, there was little variation in egg masses for small females, suggesting that females in this size class were insensitive to fluctuations in resources during reproduction. This apparent insensitivity could represent a physiological baseline (or limit) that females with a history of constraints during development are prone to maintain. With the caveat that the relationship between changes in egg mass and food availability was significant for medium sized females only. Thus, changes in the RE of medium and large *H. convergens* females translated into changes in both daily fecundity and, to some degree, egg size, whereas in *C. maculata*, essentially all changes in RE were explained by changes in daily fecundity. However, the reproductive success of small *C. maculata* females appeared more physiologically constrained; they achieved only 40-45% of the fecundity of their larger counterparts, whereas small *H. convergens* females achieved 75-90%.

Developmental time is negatively correlated with adult body size in aphidophagous coccinellids (e.g., Michaud 2000). Accordingly, larvae in the 30 min feeding treatment tended to pupate some 10-12 days later, and at much smaller body masses, than those provided *ad libitum* food access (Table 2-1). Female body size also affected trajectories of fertility (Fig. 2-3). In *H. convergens*, fertility exhibited a linear decline in females of all size classes and appeared unaffected by fluctuations in the adult food supply, although small females suffered a more rapid decline in fertility than larger ones. Although the general trend was declining fertility over time in *C. maculata* females, the fertility of small and large females demonstrated some degree of

resurgence following the restoration of *ad libitum* food, suggesting an income effect. Thus the dynamics of female fertility in *H. convergens* appeared more influenced by maternal capital than by income, whereas the opposite was true for *C. maculata*.

Coleomegilla maculata is exceptionally polyphagous among aphidophagous coccinellids (Hodek 1996) and can even develop exclusively on pollen (Lundgren & Weidenmann 2004, Michaud & Grant 2004), whereas *H. convergens* is likely to rely more heavily on aphids for both development and reproduction. In order to best exploit an aphid population cycle, a reproductive coccinellid female must convert prey into eggs as quickly as possible during a critical 'oviposition window' (Kindlmann & Dixon 2002). Females of *H. convergens* clearly had the greater capacity in this regard, with preoviposition periods generally half that of *C. maculata* females and greater RE that translated into greater daily fecundity and higher fecundity over the course of the experiment. The RE of *H. convergens* females varied much more in response to income fluctuation than did that of *C. maculata* and the fluctuations were more clearly defined and more consistently expressed across size classes of females, supporting the third hypothesis that *H. convergens* reproduction would be more sensitive to variation in the adult food supply. Thus, *H. convergens* appeared more efficient in converting income into reproductive effort (e.g., RE) than *C. maculata*, a capacity that would be adaptive in the context of greater dependency on aphid prey that occur in 'boom and bust' cycles of availability.

Figures and tables

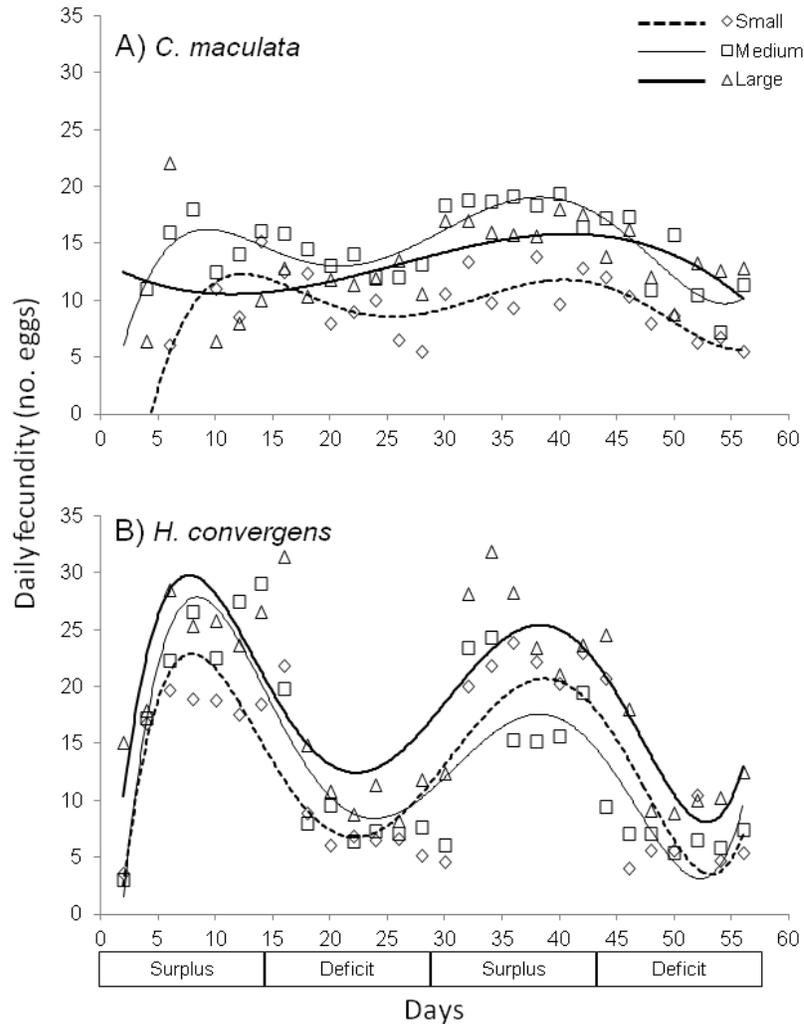


Figure 2-1 Changes in mean daily fecundity (no. eggs per oviposition day) for female *C. maculata* (A) and *H. convergens* (B) of three different sizes when provisioned for 14 day periods with *ad libitum* food (Surplus) and six hours daily access (Deficit). Females were mated and seven days old on the first day of the experiment. Female *C. maculata* were fed frozen eggs of *E. kuehniella* and female *H. convergens* were fed live *S. graminum* reared on sorghum seedlings.

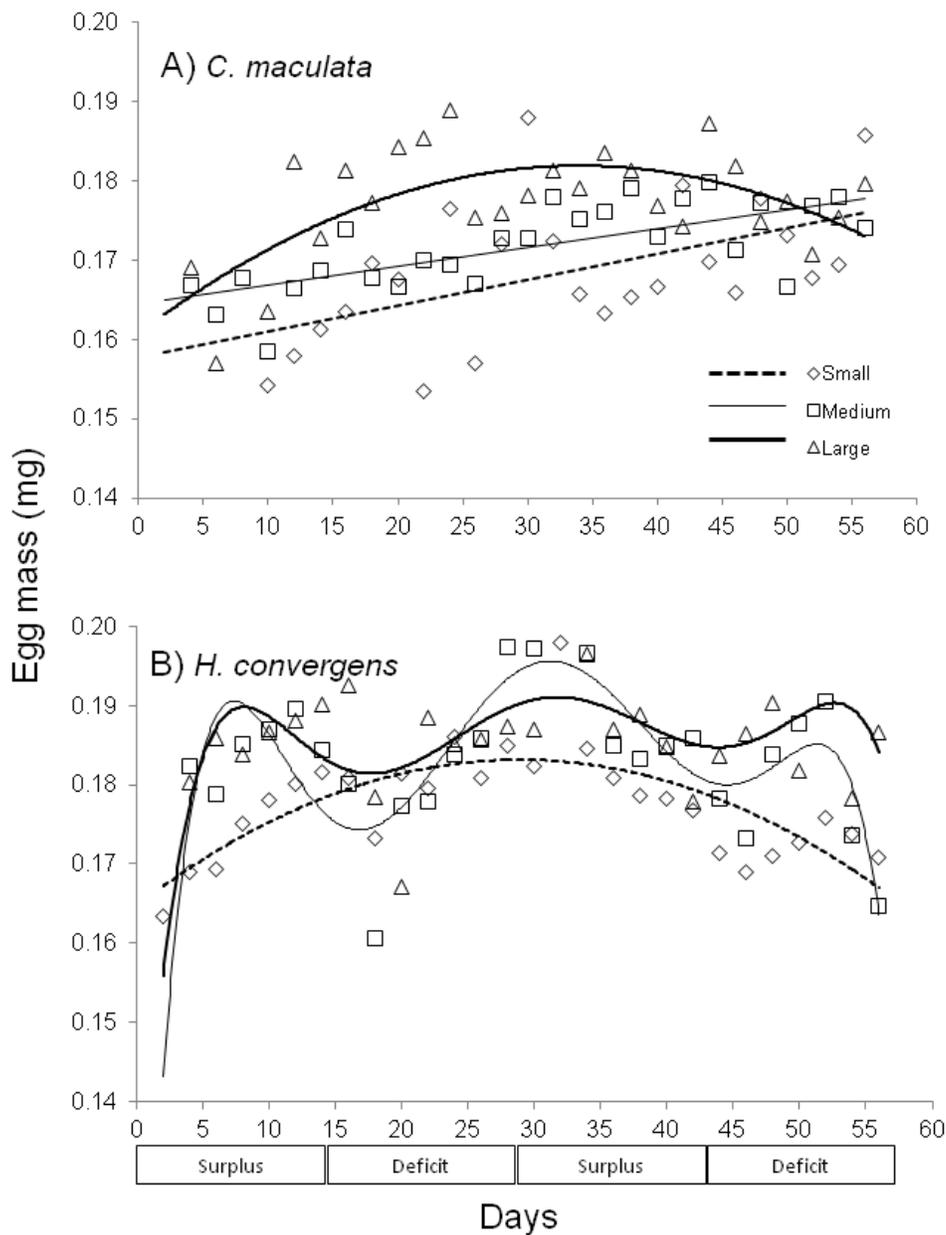


Figure 2-2 Changes in mean egg mass for female *C. maculata* (A) and *H. convergens* (B) of three different sizes when provisioned for 14 day periods with *ad libitum* food (Surplus) and six hours daily access (Deficit). Females were mated and seven days old on the first day of the experiment. Female *C. maculata* were fed frozen eggs of *E. kuehniella* and female *H. convergens* were fed live *S. graminum* reared on sorghum seedlings.

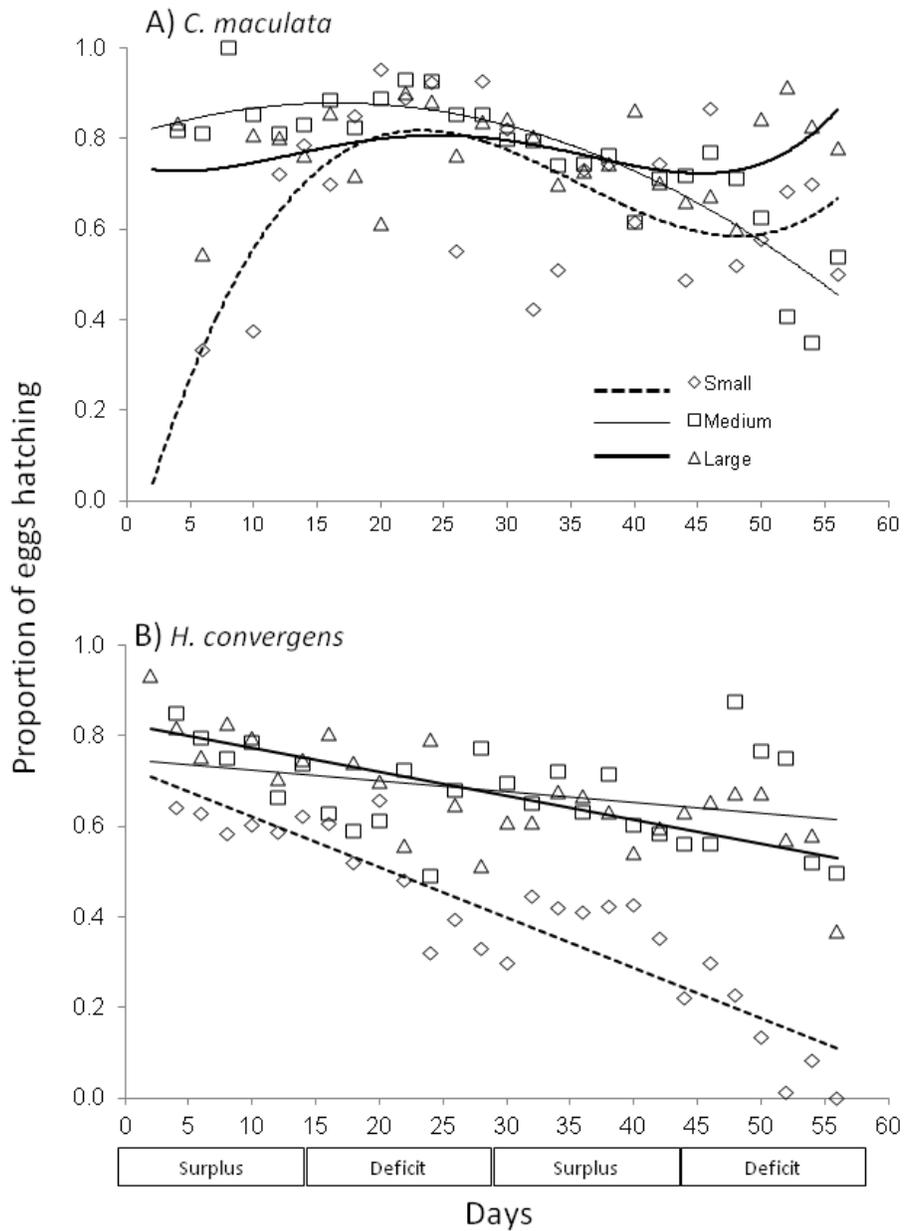


Figure 2-3 Changes in mean fertility (percent eggs hatching) for female *C. maculata* (A) and *H. convergens* (B) of three different sizes when provisioned for 14 day periods with *ad libitum* food (Surplus) and six hours daily access (Deficit). Females were mated and seven days old on the first day of the experiment. Female *C. maculata* were fed frozen eggs of *E. kuehniella* and female *H. convergens* were fed live *S. graminum* reared on sorghum seedlings.

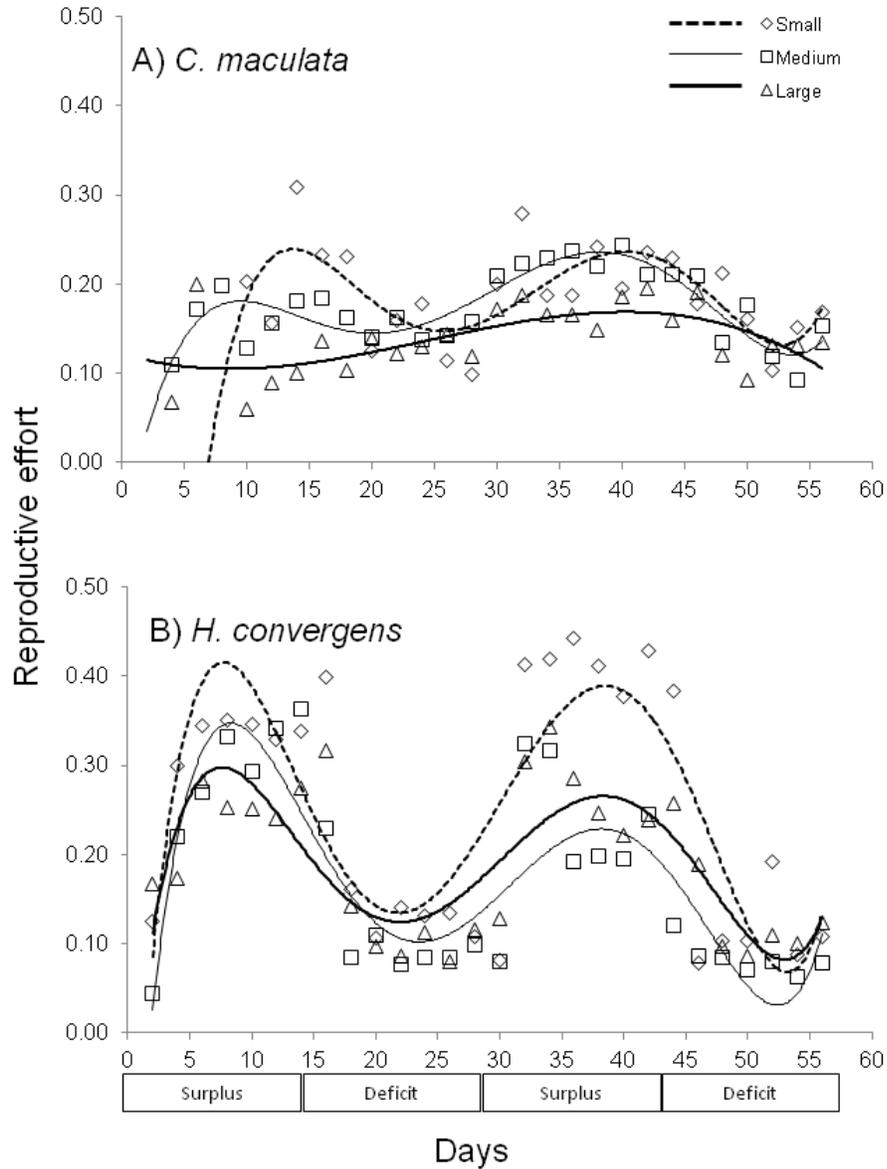


Figure 2-4 Changes in mean reproductive effort (RE = total mass of eggs / female fresh mass at emergence) for female *C. maculata* (A) and *H. convergens* (B) of three different sizes when provisioned for 14 day periods with *ad libitum* food (Surplus) and six hours daily access (Deficit). Females were mated and seven days old on the first day of the experiment. Female *C. maculata* were fed frozen eggs of *E. kuehniella* and female *H. convergens* were fed live *S. graminum* reared on sorghum seedlings.

Table 2-1 Life history data (mean \pm SE) for *C. maculata* and *H. convergens* females reared under three feeding regimes using *E. kuehniella* eggs. As adults, each female of *C. maculata* was fed *E. kuehniella* eggs, whereas *H. convergens* individuals were fed using *Schizaphis graminum*. Mated females were each confined with *ad libitum* food for their first two weeks of adult life, whereupon they were restricted to 6 hours food access per day for the next two weeks. This cycle of food access was repeated once more for a total of eight weeks of reproductive activity. Means bearing the same letter were not significantly different within rows (LSD, $\alpha = 0.05$).

Parameter	Daily larval feeding regime			df	F	P
	30 min (small)	6 h (medium)	<i>ad libitum</i> (large)			
<i>Coleomegilla maculata</i>						
Total no. females	14	13	13			
Developmental time (d)	27.3 \pm 0.7a	20.8 \pm 0.2b	17.7 \pm 0.2c	37	98.6	0.001
Adult fresh weight (mg)	9.2 \pm 0.3c	14.8 \pm 0.4b	17.2 \pm 0.4a	37	134.9	0.001
Pre-oviposition period (d)	25.4 \pm 2.8	20.9 \pm 2.7	19.9 \pm 3.0	37	0.2	0.34
Egg mass (μ g)	165.8 \pm 1.6b	169.4 \pm 1.6ab	178.2 \pm 1.4a	37	2.7	0.07
Fecundity (total eggs)	110.1 \pm 19.7b	279.2 \pm 64.2a	252.9 \pm 48.3a	37	4.3	0.02
Reproductive days	10.5 \pm 1.7	18.4 \pm 3.5	18.5 \pm 2.9	37	2.8	0.07
Daily fecundity (no. eggs)	10.1 \pm 1.0b	14.7 \pm 0.8a	13.3 \pm 0.9a	37	6.9	0.002
Fertility (% eggs hatching)	65.5 \pm 5.5	74.6 \pm 3.7	70.0 \pm 6.5	37	0.8	0.44
Reproductive effort (RE)*	2.1 \pm 0.4	3.2 \pm 0.7	2.7 \pm 0.4	37	1.1	0.31
<i>Hippodamia convergens</i>						
Total no. females	19	19	19			
Developmental time (d)	32.1 \pm 0.8a	22.8 \pm 0.5b	20.6 \pm 0.2c	54	118.6	0.001
Adult fresh mass (mg)	9.5 \pm 0.3c	15.1 \pm 0.3b	19.1 \pm 0.4a	54	205.3	0.001
Pre-oviposition period (d)	8.8 \pm 0.1b	10.1 \pm 1.6a	10.0 \pm 0.3a	54	6.1	0.004
Egg mass (μ g)	173.8 \pm 3.3b	183.6 \pm 3.3a	189.8 \pm 3.4a	54	5.9	0.004
Fecundity (total eggs)	361.3 \pm 58.4	405.8 \pm 37.3	481.8 \pm 59.1	54	1.3	0.27
Reproductive days	22.0 \pm 3.1	21.8 \pm 2.2	21.9 \pm 2.6	54	0.0	0.99
Daily fecundity (no. eggs)	16.2 \pm 1.1b	19.7 \pm 1.2a	21.6 \pm 1.1a	54	5.7	0.005
Fertility (% eggs hatching)	53.8 \pm 5.4b	69.3 \pm 3.9a	68.9 \pm 4.7a	54	3.4	0.04
Reproductive effort (RE)*	6.7 \pm 1.1	4.9 \pm 0.5	4.8 \pm 0.6	54	1.8	0.18

* Total egg mass as a proportion of initial female fresh mass = (fecundity * mean egg mass) / adult mass

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Chapter 3 - Maternal effects on offspring development as a function of female body size and age in *Hippodamia convergens*

Abstract

Maternal effects can mold progeny phenotypes in various ways but distinguishing adaptive maternal signals from physiological constraints has proven challenging. By examining the effect of laying order on progeny produced by different size classes of female ladybeetles (produced by controlling larval access to food), we show for the first time in insects that maternal signals change through adult life to synchronize progeny life histories with predictable resource dynamics, thus maximizing fitness. When fed *ad libitum* as adults, small females laid fewer, smaller eggs than large females, but laying order had a bigger impact on progeny development than maternal body size, although the two factors interacted. Later offspring of medium and large females hatched faster; these larvae fed longer in the fourth instar, pupated faster, had shorter total developmental time, and obtained greater adult mass, most notably among daughters. Laying order effects on progeny from small mothers were no significant for total developmental time and progeny mass. Only large mothers increased egg size over time and egg mass showed inconsistent correlations with developmental parameters so other, more cryptic, maternal signals impacted progeny phenotype development. Food limitation during development constrained not only fecundity and egg size, but also maternal ability to manipulate progeny phenotype, suggesting that such signals are costly. The production of faster-developing offspring that mature to larger sizes late in the oviposition cycle is adaptive for exploiting seasonal and ephemeral aphid blooms with predictable dynamics of prey abundance and predator competition.

Introduction

During the two decades since the publication of Fox and Mousseau (1998), maternal effects have received increased attention from both ecological and evolutionary perspectives (see review by Mousseau *et al.* 2009). Maternal effects are epigenetic mechanisms whereby a mother can manipulate the phenotype of her progeny in ways that may improve its fitness under prevailing or anticipated environmental conditions. Transgenerational signals guide the expression of phenotypic plasticity in offspring during development, with potentially life-long impacts on progeny life histories (Mousseau & Dingle 1991). Phenotypic plasticity is one means by which organisms may compensate for changes in environmental conditions that occur over time frames shorter than their lifespan. There are no established criteria for distinguishing 'adaptive' maternal effects from phenotypic alterations that result from environmental or nutritional constraints acting on the mother, but it is generally agreed that maternal effects can evolve and are often subject to selection, especially when the optimal progeny phenotype varies either spatially or temporally within the reproductive period of the mother (Fox & Mousseau 1998).

Shea *et al.* (2011) distinguished what they termed 'selection-based' versus 'detection-based' epigenetic mechanisms according to whether the maternal signal is fixed and independent of extrinsic factors, or depends on environmental influences experienced by the mother. Most empirical studies of maternal effects have examined the influence on progeny phenotypes of various maternal experiences such as food availability (Bashey 2006), food quality (Rotem *et al.* 2003, Bonduriansky & Head 2007, Gonzalez-Teuber *et al.* 2008), availability of oviposition sites (Gottlieb *et al.* 2011), intensity of intraspecific competition (Marshall *et al.* 2006), or temperature (Gilchrist & Huey 2001, Huestis & Marshall 2006, Gibbs *et al.* 2010, Scharf *et al.* 2010). For example, Allen *et al.* (2008) manipulated densities of the bryozoan *Bugula neritina* to demonstrate that mothers produced larger offspring under competitive, high density conditions and that larger larvae had improved survival. Less work has examined selection-based maternal effects such as changes in progeny phenotype as a function of maternal age. However, Schröder & Gilbert (2009) found that, in certain strains of the rotifer *Brachionus calyciflorus*, later-born offspring developed longer defensive spines regardless of maternal environment, a phenotype

normally induced only by exposing mothers to predation risk. Sawinksa (2004) demonstrated fixed birth order effects on offspring size in *Daphnia galeata*; later born progeny developed faster and yielded larger adults.

Insects that specialize in preying on aphids, especially lady beetles (Coccinellidae) that rely almost exclusively on aphids for larval development, face a predictable ecological scenario. At times when host plants and environmental conditions are suitable, the rapid asexual reproduction of aphids creates a bounty of resources that is available for only a short period (Dixon 2000). In as little as 2-3 weeks, host plant condition may deteriorate, a plethora of natural enemies arrive to consume the aphids, and the final aphid cohort develops into winged alatae that disperse. To obtain reproductive success on such a 'boom and bust' resource, female coccinellids must time oviposition to coincide with early stages of exponential growth in the aphid colony, a period that has been termed the 'oviposition window' (Kindlmann and Dixon 1993, 2010). Progeny produced sequentially over this period face increasingly difficult conditions as the aphid population matures, a scenario in which maternal effects could improve maternal fitness through adjustments in offspring size and developmental rate.

Egg size is one factor with the potential to affect both developmental rate and final offspring size in many animals (Fox & Mousseau 1998). In general terms, larger offspring that hatch from larger eggs may have better survival in critical early stages, develop faster, and mature into larger adults than those developing from smaller eggs (Fox 1994, Bernardo 1996). Generally, egg size tends to decrease with maternal age in most arthropods (Fox & Czesak 2000) and sometimes the advantages of larger birth size only appear under conditions of environmental stress (e.g., Harvey 1977, Ng 1988). However, size is not the only factor affecting progeny fitness at birth and other, more cryptic, maternal effects may also influence offspring quality (Bernardo 1996, Groothuis *et al.* 2005, Dloniak *et al.* 2006). For example, in the soil mite, *Sancassania berlesei*, the eggs of older females hatch later and develop more slowly independent of their size, although they eventually mature into larger offspring (Benton *et al.* 2008).

The nearctic coccinellid *Hippodamia convergens* Guerin-Meneville constitutes a good species model to test for possible maternal effects. As an important predator of many aphid species infesting cereal crops on the High Plains of the USA, it faces the ephemeral and seasonal availability of resources to promote the development of the offspring. The species is normally bivoltine in this region, one generation occurring in spring and another in fall, the adults in each

undergoing an extended period of reproductive diapause due to either winter hibernation or summer estivation, when aphids are scarce due to hot, dry conditions. Females do not begin oviposition until they encounter an aphid bloom and require 3-4 days of *ad libitum* feeding to produce their first clutch of eggs (Michaud & Qureshi 2006). Thus, any given female typically partitions her reproductive effort over a single aphid population cycle. Over the course of this cycle, larval coccinellids face increasingly intense competition for diminishing resources, as well as increasing risks of cannibalism and intraguild predation. Thus, it is possible to reason that female *H. convergens* should gradually increase egg size following the initiation of oviposition (Chapters 1, 2). Similarly, faster development would benefit later born offspring given the more imminent depletion of their food supply, and the fact that developmental time tends to be inversely correlated with adult body size in coccinellids (Honěk 1996, Michaud 2000).

The hypotheses evaluated in this chapter were: (1) that any maternal effects on egg size should serve to optimize female fitness in the context of reproduction on aphid populations that present 'boom and bust' cycles of prey availability, and (2) that female body size should mediate the trajectory of reproductive effort over time and potentially interact with maternal effects. Furthermore, by testing for maternal effects as a function of age in females of different body sizes, we distinguish between adaptive maternal effects under selection versus constraints imposed by environmental stress on the mother, through food deprivation during the larval stage, as determinants of offspring phenotype.

Materials and methods

Insect colony

Adults of *H. convergens* were collected from a sunflower field in Hays, Kansas, in June, 2009. Following transfer to the laboratory, the insects were held in a growth chamber set to L16:D8 day length and a constant temperature of $24 \pm 1^\circ\text{C}$ and relative humidity between 30 and 50%. Females were isolated in plastic Petri dishes (5.5 cm diam.) and provisioned daily with frozen eggs of the Mediterranean flour moth, *Ephestia kuehniella* Zeller, obtained from a commercial insectary (Beneficial Insectary Inc. Oak Run, California). Water was provided using a small cube of sponge. Food and water were refreshed every day. Eggs of *E. kuehniella* support normal development and reproduction in *H. convergens*, and are more convenient than aphids to provide as food for extended periods. Eggs were laid directly on the surface of the Petri

dishes and were collected daily by transferring beetles to new dishes. A new generation was produced by rearing the eclosing larvae in Petri dishes with food and water provided daily as described above. Females observed in the experiment were the F₂ generation of the animals collected in the field.

Experimental design

Eggs were separated from clusters and isolated until eclosion to prevent any egg cannibalism which has significant effects on development (Michaud and Grant 2004). Upon hatching, neonate larvae coming from 30 independent mothers ($n = 200$) were isolated in Petri dishes, randomly assigned to one of three different treatments, and reared under the same physical conditions as the beetle colony. Larvae were fed frozen eggs of *E. kuehniella* for various periods daily: i) 30 minutes per day (100 larvae), ii) 6 hours per day (50 larvae), and iii) *ad libitum* (50 larvae). Twice as many individuals were assigned to the 30 min treatment because high larval mortality was expected at this level of food deprivation.

Upon emergence, adults were weighed on an analytical balance and then isolated in a Petri dish with frozen *E. kuehniella* provided *ad libitum* and refreshed daily. Seven days after emergence, mating pairs were established and held together for the remainder of the experiment. As females began to oviposit, their eggs were collected daily. We use the term ‘daily fecundity’ to refer to the number of eggs laid in a daily bout of reproduction. We collected the 2nd, 12th and 24th oviposition days laid by females from all treatments and, using a stage micrometer under a stereo microscope (magnification x50), we measured the length and width to the nearest 0.02 mm in a sample of 12 eggs from each oviposition day. Assuming that eggs are ellipsoidal in shape, we used these measurements to estimate egg volume by the following formula for an ellipsoid (Takakura 2004):

$$\text{Volume} = LW^2\pi/6$$

A reference series of 120 eggs were collected, measured, and then weighed on a high resolution analytical microbalance to the nearest 10 μg . We then used linear regression to establish the relationship between egg volume and fresh mass, ($F_{1,145} = 1840.39$, $P < 0.0001$, $r^2 = 0.92$) that is described by the following equation:

$$\text{Mass} = 0.9171 * \text{volume} + 0.0078$$

Since the weighing of individual eggs is exceedingly time-consuming, we measured eggs under the microscope, calculated volumes, and converted values to egg mass using the equation above. Egg mass was required to estimate female reproductive effort (RE), i.e., the mass of eggs expressed as a fraction of female fresh mass at emergence.

Immediately after each egg was measured, it was isolated in a plastic Petri dish and the eclosing larva was reared on an *ad libitum* diet of *E. kuehniella* eggs. To determine time spent in each larval instar, all larvae were examined daily and all molts were recorded until pupation. As adults emerged, they were placed singly into 5 dram glass vials once they were no longer teneral. The adults were placed and sacrificed in a drying oven for 72 hours at 50°C, after which beetles were weighed individually on an analytical balance.

Statistical analysis

SAS software package was used for all analyses (SAS institute, 2008). Survival to adult emergence in the parental generation was analyzed by Chi square using PROC FREQ (SAS institute, 2008). Dependent variables for mothers and progeny (developmental time intervals, adult dry mass) were compared among treatments by one-way ANOVA using PROC GLM (SAS institute, 2008) followed by Tukey's HSD test to separate means. Comparisons of dependent variables among the 2nd, 12th and the 24th oviposition day within treatments were made using PROC MIXED for repeated measures and sphericity was tested using PROC GLM (SAS Institute, 2008). Data for progeny were analyzed first by 3-way ANOVA with treatment, oviposition and gender as independent variables to test for possible interactions of offspring gender with the other variables (results not shown). Since the only significant effect of gender was on adult mass, the mass of sons and daughters were analyzed separately. Results are shown for 2-way ANOVA's conducted with oviposition day as a repeated measure and means separated by pairwise comparisons of LS means using the Tukey-Kramer adjustment ($\alpha = 0.05$) for all dependent variables that did not fail the test for sphericity. Regressions of egg mass on the duration of various developmental stages were performed separately for different maternal body size – oviposition day groups (only significant results are reported) due to the significant effects of these factors on the dependent variables.

Results

Parental development and reproduction

Only 40% of larvae on the low food regime survived to emerge as viable adults, compared to 94% and 88% that had 6 h and *ad libitum* access to food, respectively (30 min vs 6 h: $\chi^2 = 517.0$, $P < 0.001$; 30 min vs *ad libitum*: $\chi^2 = 218.2$, $P < 0.001$). Female fresh mass increased 35% as larval food access was increased from 30 min to 6 h, and an additional 23% as it was increased to *ad libitum* (Fig. 3-1A). Increases in female body size were accompanied by corresponding decreases in developmental time (Fig. 3-1B), and increases in the number of oviposition days and daily fecundity (Figs. 3-1C, D). Larger females also trended toward shorter preoviposition periods and greater reproductive effort (RE) (Figs. 3-1E, F). Repeated measures ANOVA revealed an effect of maternal body size on daily fecundity ($F_{2,32} = 5.28$, $P = 0.010$); large females tended to greater daily fecundity. However, the effect of oviposition day was not significant ($F_{2,32} = 0.38$, $P = 0.686$; Fig. 3-2B) and neither was the maternal body size*oviposition day interaction ($F_{4,32} = 1.68$, $P = 0.178$). There was a marginally significant effect of maternal body size on egg mass ($F_{2,32} = 3.12$, $P = 0.057$) in that large females tended to lay larger eggs, but no effects of oviposition day ($F_{2,32} = 0.82$, $P = 0.448$) and no significant maternal body size*oviposition day interaction ($F_{4,32} = 1.97$, $P = 0.122$). The fresh mass of mothers at emergence was linearly correlated with their mean egg mass ($F_{2,33} = 4.93$, $P = 0.033$, $R^2 = 0.13$). The 2-way ANOVA with repeated measures for egg mass revealed a marginally significant effect of maternal body size ($F_{2,32} = 3.12$, $P = 0.057$) and no effect of oviposition day ($F_{2,32} = 0.82$, $P = 0.448$). When females were analyzed separately by treatment, there was no effect of oviposition day on egg size in small females ($F_{2,11} = 1.03$; $P = 0.389$) but egg size declined with oviposition day in medium females ($F_{2,5} = 7.88$; $P = 0.028$) and increased in large females ($F_{2,17} = 29.80$; $P < 0.0010$; Fig. 3-2A). Egg fertility (% hatching) was not affected by maternal body size ($F_{2,32} = 2.03$, $P = 0.148$), but trended lower over the course of the three oviposition days observed ($F_{2,32} = 3.48$, $P = 0.043$), and without a significant interaction between maternal body size and oviposition day ($F_{4,32} = 0.35$, $P = 0.843$). However, no declines in fertility were significant across oviposition days when size classes of females were analyzed separately (small: $F_{2,11} = 1.28$, $P = 0.315$; medium: $F_{2,5} = 3.50$, $P = 0.112$; large: $F_{2,16} = 2.38$, $P = 0.134$) (Fig. 3-2C).

Progeny development

There were significant effects of oviposition day on total developmental time, measured from oviposition to adult emergence ($F_{2,25} = 13.21, P < 0.001$), but not of maternal body size ($F_{2,31} = 0.68, P = 0.169$), and the maternal body size*oviposition day interaction was significant ($F_{4,25} = 7.17, P < 0.001$). Progeny of medium and large females reared from the 24th oviposition day tended to emerge as adults sooner than did progeny from earlier oviposition days, and a similar trend was evident, although not significant, in small females (Fig. 3-3). The percentage of progeny surviving to adult was not affected by maternal body size ($F_{2,31} = 0.16, P = 0.850$) or oviposition day ($F_{2,25} = 0.03, P = 0.968$).

There were significant effects of oviposition day on the developmental rates of various immature stages in all three treatments, with most differences evident between the 2nd and the 12th oviposition day and occurring during the egg, fourth instar, and pupal stages (Fig. 3-3). Oviposition day affected hatching time ($F_{2,25} = 48.58, P < 0.001$) but there was no effect of maternal body size ($F_{2,31} = 0.16, P = 0.854$), although the maternal body size*oviposition day interaction was significant ($F_{4,472} = 19.10, P = 0.186$). Ignoring maternal body size, eggs from the 24th oviposition day hatched faster than those from the 12th that, in turn, hatched faster than those from the 2nd oviposition day ($F_{2,27} = 51.15, P < 0.001$; Tukey- Kramer test, $\alpha = 0.05$).

There was no effect of either maternal body size or oviposition day on the duration of the first larval instar ($F_{2,31} = 0.02, P = 0.977$ and $F_{2,25} = 0.89, P = 0.423$, respectively) and the same was true for the duration of second ($F_{2,31} = 1.49, P = 0.240$ and $F_{2,25} = 0.89, P = 0.423$, respectively) and third instars ($F_{2,31} = 2.07, P = 0.142$ and $F_{2,25} = 2.63, P = 0.091$, respectively). However, oviposition day had a significant effect on the duration of the fourth instar ($F_{2,31} = 21.43, P < 0.001$), whereas maternal body size did not ($F_{2,31} = 1.24, P = 0.302$), and the maternal body size*oviposition day interaction was significant ($F_{4,25} = 3.24, P = 0.027$). Ignoring maternal body size, larvae reared from the 2nd oviposition day completed the fourth instar around one day faster than those reared from either the 12th or the 24th oviposition days, the latter groups being not significantly different ($F_{2,29} = 22.65, P < 0.001$; Tukey-Kramer test, $\alpha = 0.05$).

Pupation time was affected by both maternal body size ($F_{2,31} = 5.31, P = 0.010$) and oviposition day ($F_{2,31} = 44.12, P < 0.001$) with a significant interaction term between these parameters ($F_{4,25} = 2.82, P = 0.046$). Progeny of large females spent longer in the pupal stage

than did those of small females when reared from the 2nd and 24th oviposition days ($F_{2,241} = 4.42$, $P = 0.01$ and $F_{2,69} = 6.55$, $P = 0.002$, respectively) and longer than those of medium females when reared from the 12th oviposition day ($F_{2,153} = 8.37$, $P < 0.001$), with all other differences non-significant.

Since the largest effects of treatment on development appeared to be on hatching time, duration of the fourth instar, and pupation time, relationships among these stages were explored using linear regression. Hatching time was positively correlated with pupation time ($F_{1,470} = 14.78$, $P < 0.001$, $r^2 = 0.030$) and negatively correlated with duration of the fourth instar ($F_{1,487} = 33.49$, $P < 0.001$, $r^2 = 0.064$) with little variation explained in either case. However, there was a strong negative correlation between pupation time and duration of the fourth instar and variation in one variable explained more than a third of the variation in the other ($F_{1,470} = 304.94$, $P < 0.001$, $r^2 = 0.393$).

A 3-way ANOVA on progeny dry mass revealed a significant effect of oviposition day ($F_{2,509} = 15.86$, $P < 0.001$) and gender ($F_{1,509} = 15.86$, $P < 0.001$), but not maternal body size ($F_{2,509} = 2.01$, $P = 0.136$). However, oviposition day interacted significantly with maternal body size ($F_{4,509} = 4.24$, $P = 0.002$) but not with gender ($F_{2,509} = 1.59$, $P = 0.205$) or maternal body-size*gender ($F_{2,509} = 0.35$, $P = 0.703$) nor the three-way interaction ($F_{4,509} = 1.03$, $P = 0.391$) were significant. Because of the significant effect of gender on body size, male and female progeny were analyzed separately. The repeated measures ANOVA for male progeny revealed a significant effect of oviposition day ($F_{2,21} = 9.26$; $P = 0.001$), but no effect of maternal body size ($F_{2,30} = 0.21$, $P = 0.812$) and no significant interaction between the two ($F_{4,21} = 2.76$, $P = 0.054$). The mass of female progeny was significantly affected by oviposition day ($F_{2,21} = 14.92$, $P < 0.001$), but not by maternal body size ($F_{2,31} = 0.01$, $P = 0.987$), although the interaction between the two was significant ($F_{4,21} = 3.06$, $P = 0.039$). With the exception of small females, both male and female body size trended larger in progeny from later oviposition days, the magnitude of the increase tending to be larger in females than in males (Fig. 3-4). Egg size was positively correlated with adult body size in both sons and daughters in the early and middle oviposition days of both medium and large females (Table 3-1), but this relationship broke down in late oviposition days, except for the sons of medium females.

Egg size showed inconsistent relationships with the duration of various developmental stages across maternal size classes and the oviposition days observed (Table 3-1). For example,

egg size was negatively correlated with hatching time in the 2nd oviposition day of medium females, but positively correlated in the 12th oviposition day. Additionally, changes in egg size over time (Fig. 3-5A) did not seem associated with changes in adult size for either female or male progeny (Fig. 3-5B, C, respectively).

Discussion

Some transgenerational effects on phenotypic plasticity may lack adaptive significance and simply arise due to constraints on maternal development and reproduction, especially food deprivation (Fox & Mousseau 1998). Our experiment represents the first attempt to characterize interactions between fixed, rather than environmentally-induced, maternal effects and a physiological constraint (larval food supply). Although the *H. convergens* females in this study were reared with different resource levels, they all received *ad libitum* food as adults. Therefore, the observed maternal effects on offspring size and developmental time did not occur in response to any extrinsic cues that might signal diminishing conditions for progeny. Although there was a trend toward increasing proportional reproductive effort with increasing female body size (Fig. 3-1E), progeny phenotypes were more impacted overall by laying order than by maternal body size.

Ludwig & Fiore (1960) observed laying order effects on rate of development in the mealworm *Tenebrio molitor*; later born offspring matured more quickly, albeit with some temperature interactions, but had shorter adult lifespans. Mousseau & Dingle (1991) reviewed maternal effects on offspring quality in insects and found that for most species studied, females tended to produce their 'best' progeny early in life, with much of the variation being mediated by egg size plasticity. Similarly, Tschinkel (1993) examined laying order effects in the tenebrionid beetle *Zophobas atratus* and found that later-born females were less fecund. Mohaghegh *et al.* (1998) found that younger eggs of *Podisus maculiventris* took longer to hatch than older ones, but progeny developmental time increased with maternal age. Indeed, the general trend in insects is to produce smaller eggs later in life, rather than larger ones (Fox & Czesack 2000), possibly reflecting an adaptive response to seasonality, or constraints imposed by senescence. In contrast, our data indicate that, with the exception of low-condition individuals, *H. convergens* females tended to produce offspring of increasing quality as a function of laying order. Furthermore, although egg size correlated with adult body size in early and mid-life oviposition

days produced by medium and large females (Table 3-1), was not consistently indicative of offspring quality (Fig. 3-5), as previously recognized by Bernardo (1996).

It is possible to argue that the observed changes in offspring developmental trajectories are adaptive for the species' ecological lifestyle. *Hippodamia convergens* females face a predictable trajectory of prey availability for their progeny; a rapid increase in the food supply as aphid colonies grow exponentially, followed by an inevitable and precipitous collapse as predators and parasitoids accumulate and deplete the resource, and a final cohort of aphids develop into alatae that disperse to seek new host plants. In this context, an adaptive reproductive program for mothers is to simply produce larger, faster-developing offspring as a function of laying order. In most insects that have been studied for laying order effects, mothers invest more heavily in earlier offspring than in later ones (Mousseau & Dingle 1991), an adaptive strategy if the risk of adult mortality is relatively high and density-independent. However, cryptic maternal effects in the soil mite, *Sancassania berlese*, result in later born offspring maturing into larger adults in an ecological scenario analogous to aphidophagy; mothers face predictable deterioration of environmental conditions for later-born progeny (Benton *et al.* 2008). In contrast to aphidophagous coccinellids, in which developmental time is negatively correlated with final body size (Michaud 2000, Dixon 2000), maternal signals in *S. berlese* caused eggs of older females to hatch later and the resulting immature stages to feed longer and extend their developmental period in order to achieve greater adult size.

Despite a general lack of agreement with empirical data from other insect groups, these results are consistent with theoretical predictions. McGinley *et al.* (1987) argued that environmental variability alone is not sufficient to select for variation in offspring size and concluded that females might alter offspring phenotype in an adaptive manner if optimal offspring size changed in a predictable manner seasonally. However, their discussion focused on maternal responses to environmental cues and the degree of correlation between cues and the conditions experienced by offspring; a fixed program of adaptive change in offspring size was not considered. Fischer *et al.* (2010) argued that plasticity in offspring size is likely to be favored over a fixed strategy when environments vary stochastically and offspring survival varies between environmental states, even if actual resource availability does not vary greatly among environments, a situation not unlike aphidophagy. Kindsvater *et al.* (2010) modeled both age and maternal condition as factors affecting phenotypic variation in offspring size. When

offspring survival was density-dependent, females evolved to produce larger offspring as they aged, the effect being more pronounced in 'high quality' females.

Laying order effects were evident in all size classes of *H. convergens* females, but small females appeared constrained in their ability to effect faster overall development in later laid progeny, and were unable to increase their final size relative to those laid early. It is notable that these trends were largely achieved by changes in the timing of developmental stages, rather than being mediated by changes in egg size; only large females were able to significantly increase egg size over the course of the first 12 oviposition days (but see chapter I) and there was no consistent relationship between egg size and duration of developmental stages within treatment groups (Table 3-1) or between egg size and the final body size of the offspring produced (Fig. 3.5). Eggs laid later in a female's life hatched faster than those laid earlier, and the larvae fed for a longer period in the fourth instar, the stage responsible for most larval weight gain. Interestingly, a longer period of feeding in the fourth instar was associated with a shorter pupation time (almost 40% of variance explained in linear regression), such that the later-born progeny of females in medium to high condition benefited from faster development, larger final size, and less time spent in the vulnerable pupal stage.

The interactions between maternal body size and offspring laying order suggest that the strength of maternal signals to progeny are sensitive to maternal condition, and that the signals themselves may be resource-limited. Moreover, there was some indication that high condition females allocated signal in a gender-specific manner. The magnitude of the increase in final progeny size as a function of laying order over 24 oviposition days was generally twice as great in daughters as in sons (24.2% and 25.2% versus 14.3% and 8.3% for medium and large mothers, respectively). Assuming female fitness is more affected by body size than male via effects on fecundity, it would be adaptive for mothers in high condition to influence the final body size of their daughters disproportionately, assuming signal strength is resource-limited. Furthermore, these results indicate fitness benefits of large size for females beyond increased fecundity and egg size, as large females also exhibited greater influence over phenotype development in their offspring.

Although paternal effects were not considered in the present study, females were paired with males of similar ages from the same larval feeding regimes and it is possible that some transgenerational signals were mediated by paternity. Bonduriansky & Head (2007) showed that

high-condition mothers of the fly *Telostylinus angusticollis* produced larger eggs and larvae that developed more rapidly when on a poor diet, but that paternal contributions from high-condition fathers were required for offspring to mature to larger body sizes. Among coccinellids, previous work suggests that paternal age at mating can have a significant impact on female reproductive parameters (Singh & Omkar 2009, Omkar *et al.* 2010).

In summary, this study demonstrate for the first time in insects how an important constraint on maternal development (larval food supply) interacts with a fixed, age-specific program of maternal signals to influence progeny phenotypes. Furthermore, this study shows that maternal effects on progeny development mediated via these cryptic signals need not be linked to egg size effects; large egg size may often confer a fitness advantage in the form of larger adult size, but this effect is independent of signals influencing duration of key developmental stages and final body size that change as a function of oviposition sequence. The observed effects of maternal body size, i.e., weaker signals from low-condition mothers, are suggestive of a substantial cost to mothers of maternal signaling. The observed changes in progeny phenotypes as a function of laying order are inferred to be adaptive for a relatively specialized aphidophagous predator that encounters predictable cycles of prey availability in which conditions for progeny are initially benign, but deteriorate rapidly over time.

Figures and tables

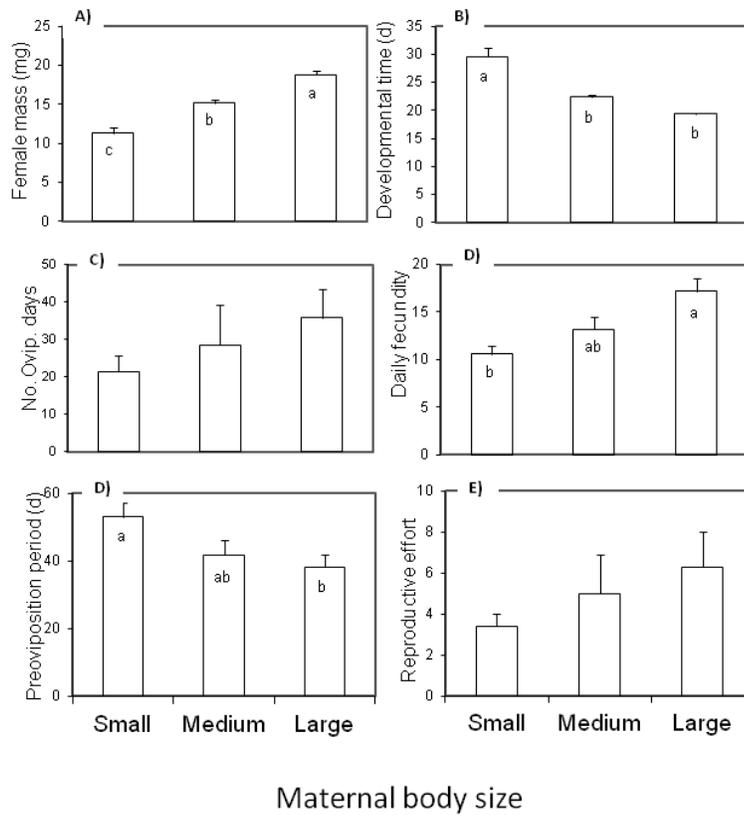


Figure 3-1 Means (+SE) of developmental and reproductive parameters for *H. convergens* females used as mothers in the maternal effects experiment. A) Female fresh mass at emergence, B) Developmental time, C) Number of oviposition days, D) Daily fecundity, E) Preoviposition period, F) Reproductive effort (RE = fresh mass of eggs / fresh mass of mother at emergence). Maternal body size was manipulated by controlling daily larval access to food (eggs of *E. kuehniella*) (small: 30 min, medium: 6 h, large: *ad libitum*). Columns bearing the same letter were not significantly different among female size classes (Tukey's HSD test, $\alpha = 0.05$).

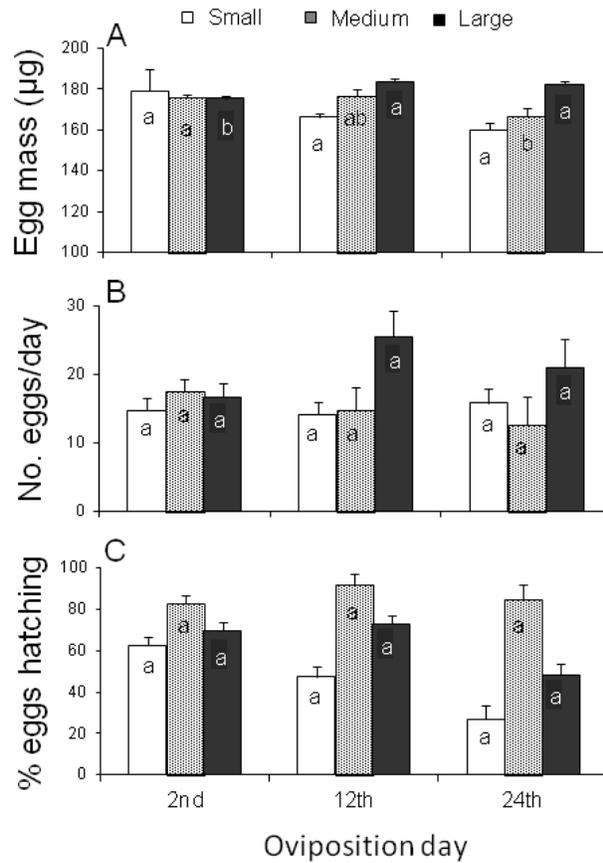


Figure 3-2 Mean (+SE) (A) Egg mass, (B) Daily fecundity, and (C) Egg fertility of three size classes of *H. convergens* females on their 2nd, 12th, and 24th oviposition days. Maternal body size was manipulated by controlling daily larval access to food (eggs of *E. kuehniella*) (small: 30 min, medium: 6 h, large: *ad libitum*). Columns bearing the same letter were not significantly different among oviposition day within female size classes (Tukey-Kramer adjustment, $\alpha = 0.05$).

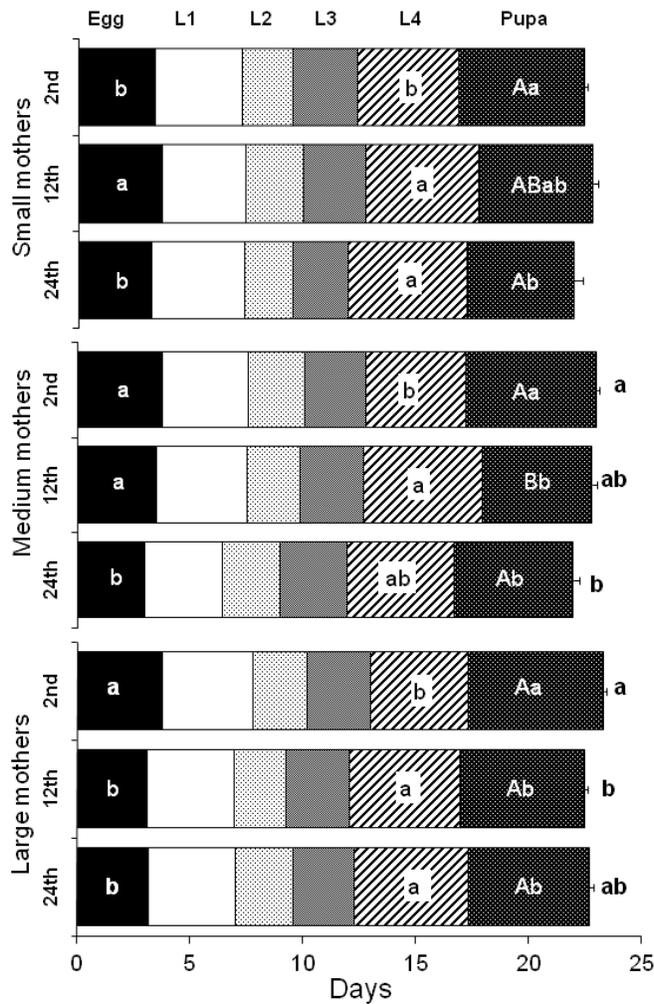


Figure 3-3 Mean durations (+ cumulative SE) of various developmental stages of *H. convergens* larvae derived from the 2nd, 12th, or 24th oviposition days of females of three different sizes produced by controlling daily larval access to food (eggs of *E. kuehniella*) (small: 30 min, medium: 6 h, large: *ad libitum*). For simplicity, only significant differences are indicated for individual life stages. Bar segments bearing different lower case letters were significantly different (Tukey-Kramer adjustment, $\alpha = 0.05$) from other oviposition days within a maternal size class. Bar segments bearing different upper case letters were significantly different among maternal size classes for a given oviposition day (Tukey's HSD test, $\alpha = 0.05$). Differences in total developmental times among progeny of different oviposition days within maternal size classes are indicated by different lower case letters to the right of each bar.

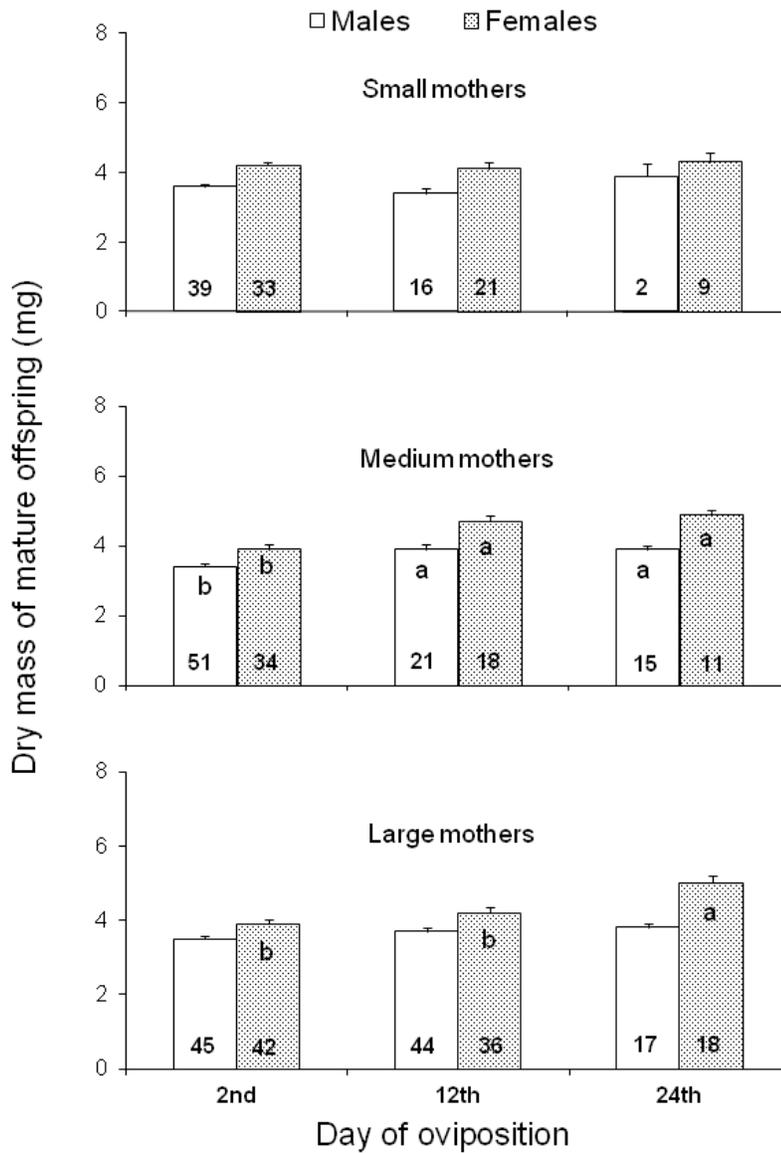


Figure 3-4 Mean dry mass (+ SE) at emergence of male and female offspring that matured from the 2nd, 12th and 24th days of oviposition by *H. convergens* females of three different sizes initially produced by controlling daily larval access to food (eggs of *E. kuehniella*) (small: 30 min, medium: 6 h, large: *ad libitum*), whereas food was provided unlimited to offspring (eggs of *E. kuehniella*). Columns bearing the same letters were not significantly different from others of the same sex and maternal size class (Tukey-Kramer adjustment, $\alpha = 0.05$). Numbers on columns reflect sample sizes.

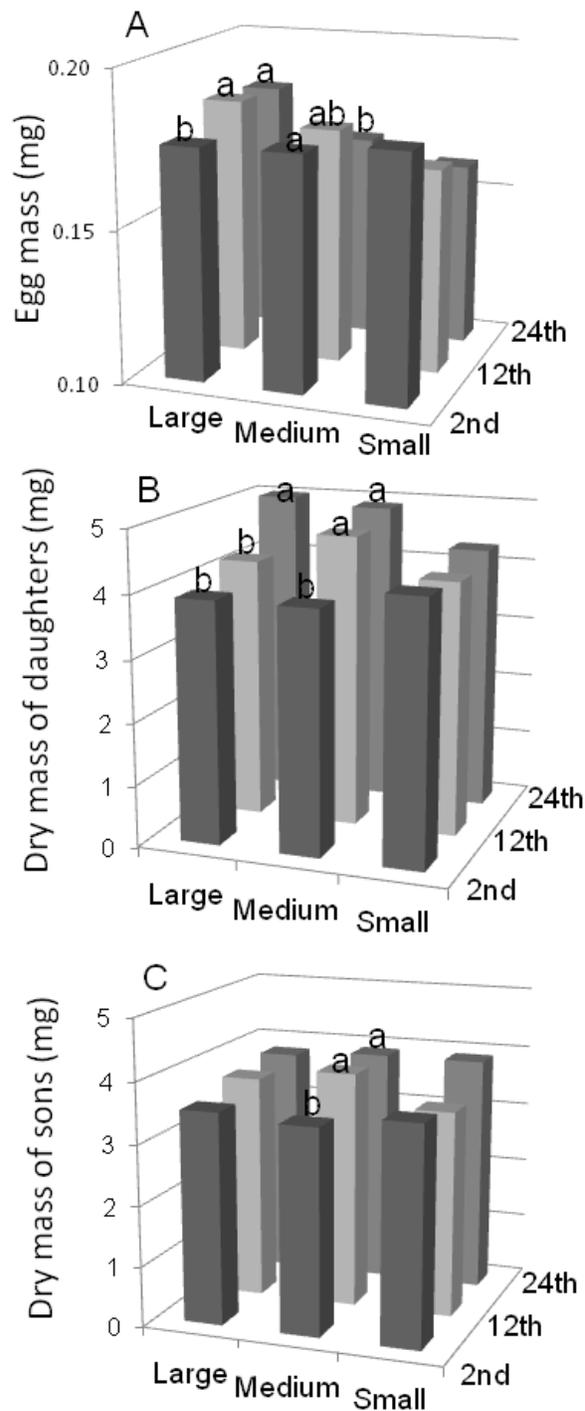


Figure 3-5 Changes in (A) mean egg size over time do not seem to be associated with changes in adult size for either (B) female or (C) male progeny. Columns bearing the same letters were not significantly different from other oviposition days within a maternal size class (Tukey-Kramer adjustment, $\alpha = 0.05$).

Table 3-1 *P* values from linear regressions of egg mass on developmental parameters in offspring of three different size classes of *H. convergens* females produced in early (2nd oviposition day), mid (12th oviposition day) and late (24th oviposition day) reproductive life. Significant positive (+) and negative (-) relationships are in bold font.

Egg weight versus:	Maternal body size		
	Small	Medium	Large
	2 nd oviposition day		
Hatching time	0.720	0.001 (-)	0.079
Duration of 4 th instar	0.140	0.013 (+)	0.333
Pupation time	0.940	0.006 (-)	0.110
Total development time	0.390	0.800	0.260
Dry mass of sons	0.850	0.030 (+)	0.001 (+)
Dry mass of daughters	0.170	0.008 (+)	0.001 (+)
	12 th oviposition day		
Hatching time	0.430	0.001 (+)	0.530
Duration of 4 th instar	0.140	0.090	0.520
Pupation time	0.870	0.660	0.043 (-)
Total developmental time	0.460	0.910	0.080
Dry mass of sons	0.004 (+)	0.001 (+)	0.002 (+)
Dry mass of daughters	0.080	0.001 (+)	0.045 (+)
	24 th oviposition day		
Hatching time	0.004 (+)	no variation	0.650
Duration of 4 th instar	0.210	0.001 (+)	0.240
Pupation time	0.970	0.130	0.080
Total developmental time	0.740	0.400	0.590
Dry mass of sons	insufficient data	0.045 (+)	0.870
Dry mass of daughters	0.680	0.470	0.470

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