Diet and social conditions during sexual maturation have unpredictable influences on female life history trade-offs

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Abstract

The trade-off between gametes and soma is central to life history evolution. Oosorption has been proposed as a mechanism by which females can redirect nutrients invested in oocytes into survival when conditions for reproduction are poor. Although positive correlations between oocyte degradation and lifespan have been documented in oviparous insects, the adaptive significance of this process in species with more complex reproductive biology has not been explored. Further, environmental condition is a multivariate state, and combinations of environmental stresses may interact in unpredictable ways. Previous work on the ovoviviparous cockroach, *Nauphoeta cinerea*, revealed that females manipulated to mate late relative to sexual maturation experience age-related loss in fecundity because of loss of viable oocytes via apoptosis. This loss in fecundity is correlated with a reduction in female mate choice. Food deprivation while mating is delayed further increases levels of oocyte apoptosis, but the relationship between starvation-induced apoptosis and life history are unknown. To investigate this, virgin females were either fed or starved from eclosion until provided with a mate at a time known to be suboptimal for fertility. Following mating, females were fed for the duration of their lifespan. We measured lifetime reproductive performance. Contrary to predictions, under conditions of delayed mating opportunity, starved females had greater fecundity, gave birth to more high-quality offspring and had increased longevity compared with that of fed females. We suggest that understanding proximal mechanisms underlying life history trade-offs, including the function of oocyte apoptosis, and how these mechanisms respond to varied environmental conditions is critical.

Introduction

Trade-offs between reproduction and longevity are a central feature of life history (Stearns, 1992; Chippindale *et al.*, 1993; Tatar & Carey, 1995; Zwaan *et al.*, 1995; Messina & Fry, 2003). We expect animals to have evolved mechanisms by which the energy requirements for reproduction and survival can be balanced to optimize fitness in a given environment, such that energy saved by a reduction in reproduction can be used

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to increase survivorship. One such mechanism is oosorption. Insects, like many animals, have the ability to degrade unfertilized oocytes and resorb their nutrients (Bell & Bohm, 1975; Chapman, 1998). This process is proposed to be an adaptive mechanism to optimize fitness in hostile environments such as parasitic attack or lack of mates, food or host substrate (Bell & Bohm, 1975; Ohgushi, 1996; Rosenheim *et al.*, 2000; Kotaki, 2003; Wang & Horng, 2004; Lopez-Carretero *et al.*, 2005; Barrett *et al.*, 2008). Under these conditions current reproduction is unlikely to be successful, so resources invested in oocytes can be recouped and reinvested into somatic functions that increase lifespan, and thus future reproductive potential (Bell & Bohm, 1975; Lopez-Guerrero, 1996; Rosenheim, 1999; Papaj, 2000; Burger *et al.*,

2004). Although correlations between oocyte degradation and improved longevity have been documented (Kaitala, 1991; Boggs & Ross, 1993; Ohgushi, 1996; Hurd *et al.*, 2001), the physiology underpinning the connection between the germline and longevity remains poorly understood (Leroi, 2001; Zera & Harshman, 2001; Barnes & Partridge, 2003; Harshman & Zera, 2006; Flatt & Kawecki, 2007; Mukhopadhyay & Tissenbaum, 2007; Hodkova, 2008).

Although oosorption to recover resources under poor conditions for reproduction is suggested to be general, studies are limited. Most previous work on the adaptive significance of oosorption has examined correlations between oocyte degradation and life history in continually reproducing, egg-laying oviparous species (e.g. Boggs & Ross, 1993; Ohgushi, 1996; Perez-Mendoza *et al.*, 2004; Osawa, 2005). Little work has been done on oosorption in females that have discrete reproductive cycles where females become 'pregnant' (ovoviviparous and viviparous). In species without maternal care, female reproductive investment ends at oviposition, whereas in species that become pregnant or provide maternal care, females must not only invest in gametes but also in their own survival until offspring are independent (Bell, 1980; Fritz *et al.*, 1982). Thus, in species that display vivipary a female must assess whether conditions would be conducive not only for embryo development but also for her own survival and decide whether to reproduce or wait.

Although the potential to facultatively resorb eggs may provide the opportunity to adaptively respond to varied environmental conditions throughout a reproductive lifetime, not all oocyte degradation need be oosorption (Barrett *et al.*, 2008). A parsimonious alternative explanation is that the loss of oocytes is simply cellular ageing, removing unused ooctyes in order to clear the reproductive tract ensuring that ovarioles are not occluded when conditions for reproduction improve (Tyndale-Biscoe & Watson, 1977) or to maintain reproductive synchrony, as in follicular atresia in mammals (Tilly, 1996). These alternatives may be especially pertinent in cyclically reproducing species. In order to fully understand the role of oocyte apoptosis, we therefore need to examine the functional significance of oocyte degradation in species with reproductive cycles and to determine under what conditions oocyte resorption confers adaptive benefits.

The ovoviviparous cockroach, *Nauphoeta cinerea*, provides a model for the study of the evolution of reproductive strategies, particularly ovarian function. Females have reproductive cycles but reproduce throughout the year and have no period of reproductive diapause (Bell *et al.*, 2007; Moore *et al.*, 2007). Within a reproductive cycle females mate, fertilize eggs and become 'pregnant', carrying the embryos in a brood pouch until the offspring are born as first-instar nymphs. Previous studies on *N. cinerea* have shown that ovarian dynamics, life history and behaviour are effected by environmental stress (Moore & Moore, 2001; Moore & Harris, 2003; Moore

& Sharma, 2005; Barrett *et al.*, 2008). Females that first have access to a mate well past the onset of sexual maturity under standard nutritional conditions have high levels of oocyte degradation via apoptosis (Moore & Sharma, 2005); a conserved form of programmed cell death where the proteins made by the cell itself initiate, regulate and execute the cell's death (Vaux & Strasser, 1996; Jacobson & McCarthy, 2002). The loss of fertilizable oocytes is correlated with a reduction in fecundity (Moore & Moore, 2001; Moore & Harris, 2003; Moore & Sharma, 2005). As females lose fecundity with a delay in mating they become less choosy, accepting copulations more rapidly during courtship (Moore & Moore, 2001). Female mate choice is important in this species as females are subjected to male enforced monogamy (Roth, 1964; Montrose *et al.*, 2004). Females mated with preferred males benefit through increased longevity (Moore *et al.*, 2003) and improved offspring quality (Moore, 1994). Reduced choosiness suggests that the costs of losing oocytes to apoptosis from a delay in mating outweigh the costs of mating with an undesirable mate (Moore & Moore, 2001; Moore *et al.*, 2007).

A quantitative genetic study revealed genetic variation for maintaining fecundity despite delayed mate availability, thus the potential to evolve long-lasting eggs exists (Moore *et al.*, 2007). So, why do fed females eliminate good eggs? One hypothesis is that alleles that cause eggs to break down in response to mating late relative to sexual maturation could be beneficial in other conditions, such as when food is scarce. We set out to test the hypothesis that the role of oocyte apoptosis observed when conditions for reproduction are poor is to recycle nutrients away from reproduction and into somatic maintenance. We did this by not allowing females to mate when first sexually mature and by depriving them of food during sexual maturation. The combination of these two conditions has been shown to influence oocyte development in *N. cinerea* (Roth & Stay, 1962) as well as stimulating increased levels of oocyte apoptosis compared with females that experience delayed mating opportunity alone (Barrett *et al.*, 2008). We predicted that the correlation between courtship behaviour and oocyte apoptosis observed in females that delay mating relative to sexual maturation (Moore & Moore, 2001; Moore & Sharma, 2005) would be similar in the starved females, with the elevated levels of ovarian apoptosis, and thus loss of fertilizable ooctyes, being reflected in a further reduction in choosiness. We further predicted, based on the hypothesis that oosorption redirects resources from reproduction into survival, that the elevated levels of oocyte apoptosis observed in females that experience a short period of starvation during sexual maturation would result in fewer offspring and a longer lifespan compared with females fed during this time. Finally, we predicted a trade-off between offspring number and quality. In all of these predictions we were wrong.

Materials and methods

Animal husbandry and experimental design

To ensure the age and virginity of all experimental animals, we removed late instar nymphs from the mass colony and sorted them by sex into two $32 \times 24 \times 10$ cm plastic containers provisioned with *ad libitum* water and food. On the day of adult eclosion, individuals were isolated and housed in $11 \times 11 \times 3$ cm plastic boxes. Females were randomly allocated to the treatments above; males to be used in the mating trials were provided with free access to food and water throughout their lives. All experimental animals were housed under standard rearing conditions of 28 °C with a 12-h/12-h reversed light/dark cycle.

Females were randomly allocated to one of two premating treatments. Females in both treatments were forced to mate late relative to sexual maturation by maintaining them as virgins for 18 days post-adult eclosion. This delay in mating is known to reduce female fecundity under standard rearing conditions (Moore & Moore, 2001). We provided females in the fed treatment with *ad libitum* rat chow and water ($n = 48$) during this pre-oviposition period. For the starved treatment, we provided females with *ad libitum* water but no food ($n = 49$) during the 18 days of sexual abstinence. Both fed and starved females become sexually receptive at 6 days post-adult eclosion (Roth & Stay, 1962). The treatment time is small compared with the life history of *N. cinerea*. Female nymphs take about 90 days to develop into adults and adult *N. cinerea* typically live for 300–400 days, with some living as long as 600 days (Moore *et al.*, 2003), and females undergoing the starved treatment still have visible fat reserves at 18 days (Barrett *et al.*, 2008). Females in both treatment groups had free access to both food and water after mating, and for the remainder of their lifespan.

Female response to courtship behaviour

We observed the courtship and copulation behaviour of females to test the effect of the premating nutritional manipulation. All females were mated at 18 days post-adult eclosion. Males were mated at 7 days post-eclosion, which is when all males are sexually mature (Roth, 1964) and producing maximum amounts of the attraction pheromone (Moore *et al.*, 1995). We placed an experimental female in a $17 \times 12 \times 6.5$ cm plastic mating arena with a randomly allocated male and observed behaviour under red light (Clark *et al.*, 1997). In *N. cinerea* courtship is characterised by a series of stereotyped events (Roth, 1964; Moore & Breed, 1986; Moore & Moore, 1988; Clark *et al.*, 1997) and as in these previous studies of mate choice we measured the time it took for a female to approach the male, the time from female approach to male wing raise (a measure of male

willingness to court) and from male wing raise to the start of copulation (a measure of female choosiness). If a female refused to mate with a male within 20 min from the start of the mating trial, she was placed back into her individual container and marked as unreceptive. In contrast to previous studies (Moore, 1990), we allowed courtship to continue for 20 min to confirm that females were unreceptive to mates. Once copulation was complete, we returned the mated females back into their individual containers. After mating trails all females (both mated and unreceptive) were provided with *ad libitum* food and water and returned to standard rearing conditions. Log linear regression was utilised to investigate whether there were differences in female receptivity (number of mated females) between the treatments. We used analysis of variance (ANOVA) to test whether measures of courtship and copulation duration differed between diet manipulation groups.

Female reproductive success and longevity

We checked mated females daily for offspring to test for differences in lifetime reproductive success. When females produced a clutch, we separated the mother and offspring and chilled the offspring at 5 °C for 2 h. This renders the nymphs immobile whilst not being permanently damaging. We recorded the date of birth and number of offspring produced for all clutches. Offspring were then maintained to adulthood.

We tested the influence of diet treatment on the number of offspring produced in each of the first four clutches using ANOVA. Too few females produced a 5th clutch to perform a meaningful analysis (four starved, one fed). Levels of significance were Bonferroni corrected to account for the use of multiple tests to test the single hypothesis (Rice, 1989; Perneger, 1998; Cabin & Mitchell, 2000) that diet during sexual maturation would have an effect on female lifetime reproduction (Moore & Moore, 2001; Moore & Harris, 2003). Previous work shows that the timing of parturition of the first clutch is an important predictor of lifetime reproductive success in females that delay mating in the presence of food (Moore *et al.*, 2007). Consequently, we investigated whether there were differences in the reproductive schedule between the diet manipulation groups, and tested for a relationship between timing of reproduction and fecundity. We analyzed timing of parturition using a Kaplan–Meier survival analysis; significant differences in timing of parturition between diet manipulations and the relationship between time to reach parturition and fecundity were examined using the Cox Regression Tarone–Ware log rank test.

We predicted that we would observe either a decrease in fecundity with an increase in lifespan, or no change in longevity and a recovery in post first clutch fecundity based on previous evidence for oosorption in poor reproductive conditions from continuously reproducing

(synovigenic) insects (Kaitala, 1991; Boggs & Ross, 1993; Chippindale *et al.*, 1993; Ohgushi, 1996; Wang & Horng, 2004). We therefore checked boxes containing mated females daily for mortality and recorded the date of death. We calculated longevity as adult lifespan (adult eclosion to death) and used Cox Regression Mantel log rank test to investigate the difference in female longevity between diet treatments.

Offspring quality

We measured neonatal size and mass, development time, juvenile survival and size upon adult emergence as parameters of offspring quality to investigate the relationship between offspring quality and quantity. Measures were taken for offspring from the first and second clutch only. We examined offspring from the first and second clutches to separate the direct effects of food availability during development of the oocytes of the first clutch and any indirect effects that may persist despite both manipulation groups having free access to food throughout oocyte development for the second clutch.

We took photographs of newly eclosed nymphs using a Leica MZ12.5 dissecting stereomicroscope fitted with a SPOT Insight camera (Diagnostic Instruments, Inc., Sterling Heights, MI, USA), and measured the pronotum width using ImageJ V.1.29X (http://rsb.info.nih.gov/ij) to measure neonatal offspring size. We measured mean neonatal offspring mass by weighing the entire clutch with an Ohaus analytical balance and dividing by the number of nymphs. We then transferred the neonates to a 17 × 12 × 6.5 cm plastic family box with *ad libitum* food and water and monitored the boxes daily for adult eclosion. We recorded the date of emergence, sex and pronotum width for each newly emerged adult. The total number of nymphs surviving to adult eclosion was also recorded.

We used linear mixed effect models to test the effect of maternal sexual maturation diet (independent variable) on neonatal offspring size (dependent variable). Family was nested within diet as a random effect to control for genetic and maternal effects. Males develop more quickly than females in this species so we analyzed development time separately for each sex. We also performed separate analyses for the offspring of the first and second clutch. We used ANOVA to test for influence of the sexual maturation diet on mean neonatal offspring mass (mass of clutch/number in clutch). We used ANCOVA to look at the confounding effect of the time to parturition on neonatal offspring mass in both treatment groups; we then performed linear regressions to look at the different slopes of this relationship in the two diet manipulation groups.

We used Cox regression to test the effect of maternal diet during a delay in mating (independent variable) on offspring juvenile development time (dependent variable). Juvenile development time was calculated for each clutch as the geometric mean of the time from birth to eclosion. We used geometric means as opposed to arithmetic means as there was great variability in the clutch sizes produced by different females, and the geometric mean is a more accurate measure of central tendency when data may change in a relative, as opposed to additive, fashion (McAlister, 1879).

We calculated percentage juvenile survival for each clutch and compared survival rates using Welch's approximate *t*-test, which accounts for nonequal variance between groups, to investigate the effects of maternal diet during a delay in mating on juvenile mortality. We calculated the geometric mean of adult offspring size per clutch, and we then used ANOVAs to test for size differences in adult offspring between maternal diet groups.

Results

Female response to courtship behaviour

Contrary to our prediction, the mating behaviour of starved females did not differ from that of fed females under conditions of when both had been deprived of mates when first sexually receptive, despite the differences in ovarian physiology seen between diet manipulation groups in the study by Barrett *et al.* (2008) under the same experimental conditions. Fed and starved females were equally likely to mate (trials resulting in no mating: fed 4/48, starved 9/49; Log linear regression, $\chi_{1,96} = 2.154$, $P = 0.142$). There was no significant difference between fed and starved females when they were first given a male in the time taken for the female to approach the male, the onset of male courtship (male wing raise), the initiation of mating (courtship duration) or subsequent copulation duration (Table 1).

Female reproductive success and longevity

Starved females had greater lifetime reproductive success than fed females under conditions of delayed mating opportunity (Fig. 2). Of the females that mated, 100% of

Table 1 Effect of female sexual maturation diet and delayed mating opportunity on the courtship and copulation of *Nauphoeta cinerea*.

Character	Treatment	Mean time (s) ± SD	F	d.f.	P	
Approach	Fed	31.2 ± 40.7	0.065	1, 82	0.799	ns
	Starved	30.0 ± 36.9				
Wing raise	Fed	77.5 ± 186.0	0.159	1, 82	0.692	ns
	Starved	38.0 ± 46.5				
Courtship duration	Fed	182.7 ± 254.6	1.681	1, 82	0.198	ns
	Starved	269 ± 356.3				
Copulation duration	Fed	874.6 ± 81.46	1.095	1, 82	0.298	ns
	Starved	854.8 ± 91.7				

Table 2 Effect of female sexual maturation diet and delayed mating opportunity on timing of birth and fecundity in *Nauphoeta cinerea*.

Clutch	Treatment	Time to parturition				Number of offspring			
		Mean (days) ± SD	χ†	d.f.	P	Mean ± SD	F‡	d.f.	P
1	Fed	83.4 ± 30.9	8.222	1, 72	0.007	18.2 ± 8.3	13.457	1, 72	< 0.001
	Starved	66.5 ± 10.7				27.5 ± 8.4			
2	Fed	53.0 ± 10.5	2.262	1, 64	0.133	23.6 ± 9.2	0.019	1, 64	0.891
	Starved	50.0 ± 4.7				23.9 ± 9.3			
3	Fed	48.7 ± 17.8	0.070	1, 45	0.791	18.7 ± 8.2	0.337	1, 45	0.564
	Starved	48.5 ± 3.8				20.0 ± 9.4			
4	Fed	45.3 ± 2.1	0.070	1, 15	0.791	20.0 ± 8.6	1.332	1, 15	0.266
	Starved	48.5 ± 4.4				14.2 ± 8.8			

†Using Cox regression.
‡Using ANOVA.

the starved females produced offspring, whereas 75% of the fed females reproduced (Log linear regression, $\chi_{1,84} = 12.845$, $P < 0.001$).
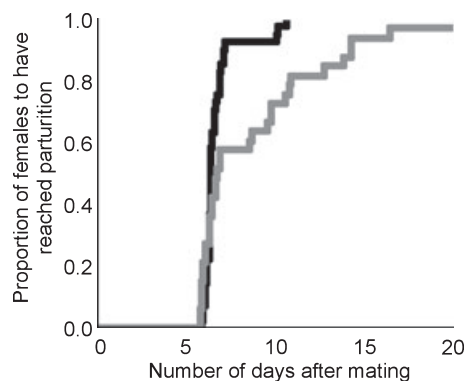
Females that were starved premating took less time to give birth to their first clutch than females that had continued access to food (Table 2, Fig. 1). Timing of parturition and number of offspring in the first clutch were related. Females that gave birth later had fewer offspring (Cox regression, $t_{1,74} = 2.038$, $P = 0.042$). There was no significant effect of treatment on the inter-clutch time between any other clutches (Table 2).

Of the females that reproduced, starved females produced significantly more offspring in their first clutch than fed females even with Bonferroni adjusted levels of significance (Fig. 2a, Table 2). The positive effect of starvation throughout the premating period on fecundity in the first clutch was not simply a result of an association with timing of parturition; among females that gave birth to a first clutch with standard timing for *N. cinerea* (Moore *et al.*, 2007), starved females had greater fecundity of fed females under conditions of delayed mating opportunity

(Fig. 2b; ANOVA, $F_{1,56} = 21.392$, $P < 0.001$). There were no significant differences between treatment groups in the number of offspring in the subsequent clutches (Table 2).

Females that were starved and did not mate when first sexually mature had more clutches (Fig. 2c; ANOVA, $F_{1,84} = 13.457$, $P < 0.001$). The combination of greater first clutch fecundity and additional clutches resulted in these females having almost twice the lifetime reproductive success than females that had continued access to food and did not mate when first sexually mature (Fig. 2d; ANOVA, $F_{1,84} = 20.59$, $P < 0.001$).

Counterintuitively, starved females that did not mate when first sexually mature had increased longevity compared with fed females (Fig. 3, Cox regression, $\chi_{1,74} = 4.06$, $P = 0.025$), despite the increased lifetime reproductive success of starved females. An analysis of covariance with longevity as the covariate revealed that the higher lifetime reproductive success seen in starved females was not simply a function of increased lifespan, but of the diet manipulation treatment (ANCOVA, $F_{1,70} = 14.968$, $P < 0.001$).

Offspring quality

Measures of offspring quality at birth showed that females that were starved when deprived of a mate when first sexually receptive had larger but lighter neonatal offspring in their first clutch than fed females (linear mixed effect model, Table 3). Although starved females had larger offspring, their neonatal offspring had lower mean mass (ANOVA; Table 3).

More offspring born to starved females from both the first and second clutch survived to adulthood (Fig. 4). This was true for both the offspring in the first clutch (Fig. 4a; Welch's approximate *t*-test, $t_{1,38.346} = 2.386$, $P = 0.022$) and in the second clutch (Fig. 4b; Welch's approximate *t*-test, $t_{1,36.203} = 20.023$, $P = 0.051$). Offspring of both sexes born in the first clutch to females that were starved during the period of delayed mating opportunity developed more slowly than those born to
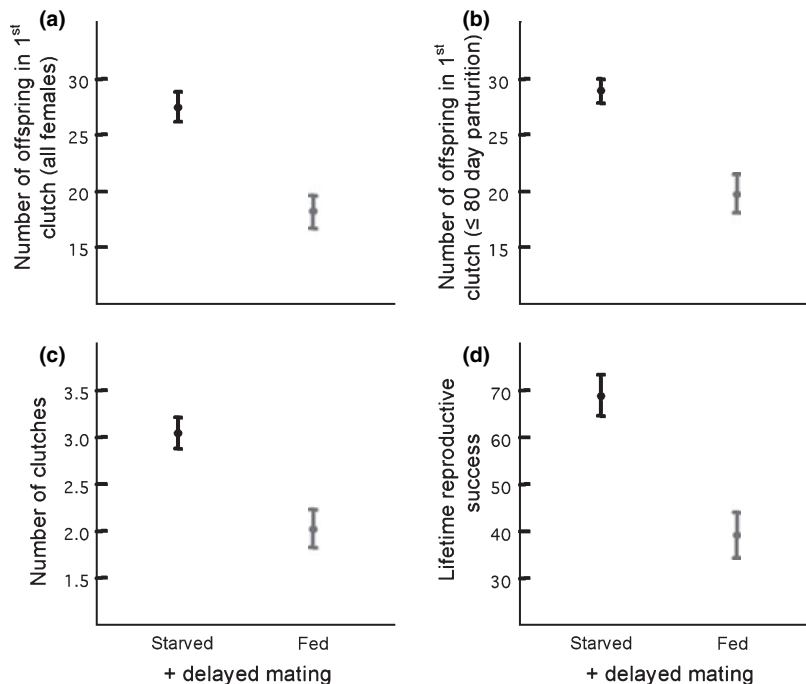

Fig. 1 Effect of delayed mating opportunity and diet during sexual maturation on schedule of reproduction. Females starved during the period of delayed mating opportunity ($N = 41$, *black line*) give birth to their first clutch faster than females that were fed throughout ($N = 33$, *grey line*).
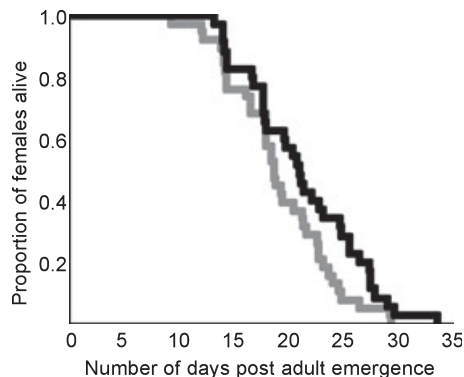
Fig. 2 Effect of delayed mating opportunity and diet during sexual maturation on reproduction. (a) Number of offspring in the first clutch of all females. Females that were starved during the period of delayed mating opportunity produced significantly more offspring (starved mean = 27.46, SD = 8.44; fed mean = 18.18, SD = 8.28). (b) Number of offspring in the first clutch of females with normal ($\leq$ 80 days) parturition. Females that were starved during the period of delayed mating opportunity produced significantly more offspring (starved mean = 28.89, SD = 6.82; fed mean = 19.74, SD = 7.49). (c) Total number of clutches. Females that were starved during the period of delayed mating opportunity had more clutches (starved mean = 3.05, SD = 1.05; fed mean = 2.02, SD = 1.37). (d) Lifetime reproduction. The end result was that females that were starved during the period of delayed mating opportunity had greater lifetime fecundity (starved mean = 68.88, SD = 28.45; fed mean = 39.02, SD = 31.95).

fed females (Table 4). In the second clutch, however, there were no significant effects of diet manipulation on development time in either sex (Table 4). There were no

Fig. 3 Effect of delayed mating opportunity and diet during sexual maturation on longevity. Females that were starved during the period of delayed mating opportunity (N = 35; *black line*) lived significantly longer than those that were fed throughout (N = 38; *grey line*).

significant effects of maternal diet on the adult size of male or female offspring in either clutch (Table 4).

Discussion

Contrary to our predictions we found no differences in mate choice, despite the known differences in ovarian physiology (Barrett *et al.*, 2008). Further, we found that females starved during the period of delayed mating opportunity were more fecund both in terms of early-lifetime fecundity and lifetime reproductive success, and had offspring of higher quality, than females that had continued access to food throughout sexual maturation. Moreover, females that were starved during sexual maturation also lived longer than fed females. Our results show that *ad libitum* food in the absence of males can have a negative impact on both reproduction and survival. Rather than acting synergistically with mate deprivation, food deprivation appears to ameliorate the effect of not mating at the optimal time, perhaps because the latter involves physiological pathways that are beneficial when there are no mates available. Thus, our results challenge our concept of what constitutes good and poor conditions for reproduction and how these

Table 3 Effect of female sexual maturation diet and delayed mating opportunity on neonatal offspring size and mass in *Nauphoeta cinerea*.

Clutch	Treatment	Neonatal mass				Neonatal size			
		Mean (mg) ± SD	F†	d.f.	P	Mean (mm) ± SD	F‡	d.f.	P
1	Fed	7.30 ± 1.50	3.294	1, 63	0.053	1.16 ± 0.07	7.489	1, 127.93	0.007
	Starved	6.64 ± 0.78				1.20 ± 0.06			
2	Fed	3.70 ± 1.10	0.198	1, 64	0.658	1.25 ± 0.05	0.835	1, 123.5	0.363
	Starved	3.63 ± 1.13				1.24 ± 0.08			

†Using ANOVA.
‡Using a linear mixed effect model for neonatal size with family nested under diet manipulation as a random effect.

conditions can affect the allocation of resources between reproduction and survival. Environmental condition is a multivariate state, and the nature of interactions between influences cannot be assumed or ignored.
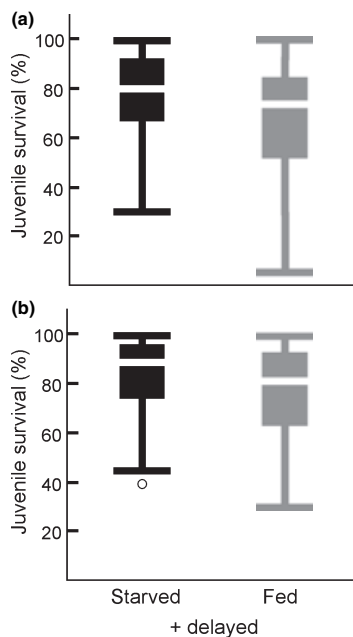
The absence of food during a manipulated delay in mating did not affect female response to male courtship behaviour

We predicted that we would observe a decrease in the amount of courtship required for starved females compared with fed females under conditions of delayed mating opportunity. This prediction was based on previous work showing fed females that delay mating have high levels of oocyte apoptosis (Moore & Sharma, 2005). In fed females, oocyte apoptosis is correlated with a reduction in fecundity (Moore & Moore, 2001; Moore & Harris, 2003) and a reduction in female choosiness (Moore & Moore, 2001). Levels of ovarian apoptosis are further elevated in females that are starved during the period of delayed mating opportunity (Barrett *et al.*, 2008). Consequently, we made the parsimonious, but wrong, prediction that females would respond to the threat of a further loss of fecundity by a becoming even less choosy. However, starved females did not regain choosiness with the recovery of fecundity. This raises an interesting question as to what proximal mechanisms might govern mate choice behaviour. It may be that investment in mate choice is governed by social experience rather than ovarian physiology. This highlights a problem when analysing life history characteristics. It is not clear how often trade-offs observed at physiological and evolutionary levels involve the same proximal mechanisms (Stearns, 1992; Flatt & Kawecki, 2007).

Fig. 4 Effect of delayed mating opportunity and diet during sexual maturation of mothers on offspring survival to adulthood. Survival was greater in offspring born to females starved during the period of delayed mating opportunity. (a) Survival of offspring from the first clutch. More offspring from females that were starved during the period of delayed mating opportunity survived to adulthood (fed median = 71.8%, starved median = 80%). (b) Survival of offspring from the second clutch. The same pattern was observed (fed median = 90%, starved median = 92.9%).

The absence of food during a manipulated delay in mating had a positive direct effect on female fecundity and lifespan

Contrary to our predictions, starved females had almost double the lifetime reproductive success of fed females when both delayed mating. Why did starved females regain their fertility? The observation that fed females had protracted reproductive schedules under delayed mating opportunity is intriguing. Females in this experiment displayed a similar pattern of reproduction observed in a prior quantitative genetic study of reproductive potential following a delay of mating (Moore *et al.*, 2007). In that study, fed females that delayed mating exhibited a bimodal distribution of parturition times. Females that produced their first clutch with normal timing had much greater reproductive success than the group that had extended preparturition time for their first clutch. The timing of parturition of the first clutch was both a good predictor of reproductive outcome and highly heritable. In the present study nearly all starved females had normal preparturition times

Table 4 Effect of female sexual maturation diet and delayed mating opportunity on offspring development time and adult size in *Nauphoeta cinerea*.

Clutch		Treatment	Developmental time			Adult size			
			χ†	d.f.	P	Mean (mm) ± SD	F‡	d.f.	P
1	♀	Fed	4.410	1, 63	0.036	6.34 ± 0.176	3.230	1, 63	0.077
		Starved				6.25 ± 0.19			
	♂	Fed	9.478	1, 63	0.002	6.07 ± 0.27	0.623	1, 63	0.433
		Starved				6.02 ± 0.23			
2	♀	Fed	0.225	1, 53	0.635	6.33 ± 0.13	0.312	1, 53	0.579
		Starved				6.31 ± 0.14			
	♂	Fed	0.022	1, 54	0.882	6.02 ± 0.15	0.036	1, 54	0.851
		Starved				6.01 ± 0.14			

†Using Cox regression.
‡Using ANOVA.

and, concordantly, greater first clutch fecundity. Starvation in the premating period appears to overcome the effects of delayed mating opportunity on timing of parturition.

This raises the question of why starved females take less time to reach parturition. The observed difference in reproductive schedules might be explained by the time it takes to 'reset' the ovary. The developmental stage at which oocyte apoptosis takes place differs among fed and starved females under conditions of delayed mating opportunity (Barrett *et al.*, 2008). Starved females invest less in oocytes (Roth & Stay, 1962; Barrett *et al.*, 2008) and their oocytes undergo apoptosis at earlier stages of development compared with the oocytes of fed females (Barrett *et al.*, 2008). By initiating apoptosis at an earlier stage of vitellogenesis, females lacking proper nutritional signals may achieve a 'head start' by both having begun the resetting process earlier and in having less to recoup from the smaller, less developed oocytes before initiating the maturation of the next set. For fed females the switch to nutrient reallocation may not have occurred. Rather, oocyte apoptosis may be a reflection of cellular ageing that reduces oocyte quality. Thus, the reduction in offspring quality may be the result of developing from older, poor quality oocytes.

Our most unexpected finding was that, despite having had more offspring, starved females that did not mate when first sexually mature lived longer than those that had *ad libitum* access to food. Previous studies on oosorption (Kaitala, 1991; Boggs & Ross, 1993; Ohgushi, 1996) and caloric restriction have shown that decreased nutritional income can increase longevity in a variety of organisms (fruit flies, Chapman & Partridge, 1996; rodents, Weindruch, 1996; yeast, Jiang *et al.*, 2000; nematodes, Walker *et al.*, 2005). This increase in lifespan reflects the process whereby restricting caloric intake down-regulates reproduction and diverts resources into somatic maintenance to increase lifespan, so that the organism may live to reproduce once the famine is past

(Kirkwood & Rose, 1991; Shanley & Kirkwood, 2000; Kirkwood, 2005). Our findings do not fit this model. Even independent of lifespan, lifetime reproductive success was greater in starved females. Although this can be partially explained by longer preparturition of fed females, among females with normal preparturition, starved and mate-deprived females were still more fertile than fed and mate-deprived females. This suggests that the costs of producing the first clutch are much greater for females that had continued access to food under conditions of delayed mating opportunity. This might reflect the costs of maintaining reproductive state as mating and food both stimulate juvenile hormone (JH) production in *N. cinerea* (Roth & Stay, 1962; Roth, 1964). JH has been proposed to be central in mediating the balance between somatic maintenance and reproduction in insects (Tatar & Yin, 2001).

The absence of food during a manipulated delay in mating had a positive indirect effect on offspring quality

As resources are limited in natural environments, a trade-off between offspring quantity and quality is expected to occur (Lack, 1954; Smith & Fretwell, 1974; Haig, 1990; Wright *et al.*, 1998; Sinervo, 1999; Sinervo *et al.*, 2000). Thus, we might predict that fed females would compensate for lower offspring numbers with higher offspring quality. However, the offspring from both the first and second clutches of females starved prior to mating had higher juvenile survival rates than those born to fed females. Thus, the effect on offspring quality as measured by survival appears to reflect a change in reproductive physiology indirectly elicited by the absence of food when coupled with delayed mating, rather than a direct effect of resource availability during oocyte maturation. Food deprivation during a delay in mating appears to alleviate the cost of delayed mating, as females under these conditions gave birth to an increased

number of more robust offspring compared with fed females that delayed mating.

Two wrongs sometimes make a right: defining conditions under which oocyte resorption is adaptive may be complex

Insects (Bell & Bohm, 1975; Lopez-Guerrero, 1996; Rosenheim *et al.*, 2000) and other animals (e.g. Kumara Pillai & Subramoniam, 1984; Hinch, 1992; Friedel, 1993; Vaschenko *et al.*, 1997; Blackburn, 1998) have the ability to resorb oocytes when conditions for reproduction are poor. The widespread assumption has been that this is an adaptive mechanism to facilitate the recycling of nutrients invested in oocytes that will not be used, reallocating these resources into survival when opportunities for reproduction are limited (Bell & Bohm, 1975; Papaj, 2000). For example, in butterflies, a reduction in food availability leads to an increase in oocyte resorption, a decrease in fecundity, but not a reduction in lifespan (Boggs & Ross, 1993). These and other similar findings suggest a direct trade-off between reproduction and lifespan mediated by the recycling of nutrients invested in oocytes that cannot be used.

Our data present a more complex picture of life history trade-offs when conditions for reproduction are poor. Although resource allocation may provide a direct connection between reproduction and survival in some species (Boggs & Ross, 1993; Ohgushi, 1996), our understanding of how this trade-off is actually mediated will depend on a fuller picture of the physiological and molecular mechanisms underlying both the decision to resorb oocytes and the mechanisms by which these resources can be reinvested into survival. In *N. cinerea* mating at a suboptimal time relative to sexual maturation elicits oocyte death (Moore & Moore, 2001; Moore & Sharma, 2005), but not the reallocation of resource. Only the combination of mate and food deprivation is sufficient to initiate the pathway leading from oocyte death to increased longevity.

The observation that ovarian apoptosis does not appear to confer any advantage to fed females that are manipulated to delay mating may reflect past selection, or lack thereof. It is likely that conditions where food is available, but mates are not, are rare. The improved reproductive outcome for starved females that do not mate when first sexually mature can be interpreted as an adaptive response to overall poor conditions for reproduction. Future research into both the proximate mechanisms behind life history trade-offs, and whether combinations of environmental conditions can lead to an uncoupling of reproduction and longevity trade-off will be fundamental in our understanding of the internal mechanisms behind the costs of reproduction and how they are connected to the external environment (Barnes & Partridge, 2003; Flatt & Promislow, 2007). This research clearly has to include organisms that reproduce cyclically as well as continuously.

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