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Maternal age affects offspring lifespan of the seed beetle, *Callosobruchus maculatus*

C. W. FOX†, M. L. BUSH and W. G. WALLIN

Department of Entomology, S-225 Agricultural Science Center North, University of Kentucky, Lexington, KY 40546–0091 USA

Summary

1. Offspring from older parents often have shorter adult lifespans than offspring of younger mothers. We examine the effects of offspring genotype, maternal age and paternal age on offspring survival, development and adult lifespan in the seed beetle, *Callosobruchus maculatus*.

2. Females took about a quarter of a day longer to develop from an egg to an adult and lived ≈7 days longer than males. Mortality patterns were best described by a logistic mortality model, and all three model parameters differed significantly between the sexes; females had a higher baseline mortality rate than males but the mortality rate increased more slowly in females than in males. Females also showed a delay, relative to males, in the age at which mortality became age-dependent.

3. The proportion of eggs that hatched and larval survivorship both declined with increasing maternal age, while egg-to-adult development time increased substantially. Contrary to the pattern observed in many other organisms, offspring of older mothers lived *longer* than offspring of younger mothers, even after controlling for heterogeneity among families. There was no evidence that paternal age affected any offspring traits.

4. The effect of maternal age on offspring lifespan was greater for male offspring than for female offspring (consistent with the general observation that the genetic and environmental factors affecting lifespan differ between the two sexes) and varied among sire families (indicating that offspring genotype mediated the non-genetic effect of maternal age on lifespan).

Key-words: Longevity, maternal effect, mortality rate, paternal effect

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Introduction

The phenotype of an individual is influenced not only by its genes and the environment in which it is raised, but also by the phenotype of its parents and the environment in which its parents are raised (Mousseau & Fox 1998). These ‘maternal effects’ (often called ‘parental effects’) are often a consequence of variation in propagule size (e.g. egg size, Fox, Thakar & Mousseau 1997) or factors other than DNA packaged inside propagules, such as maternally derived messenger RNA or proteins (Mousseau & Fox 1998). The relative influence of maternal effects on offspring phenotypes generally decline with increasing offspring age – early development and survival tend to be highly affected by propagule size and composition, while traits expressed late in development or postmaturity are not (e.g. Fox 1997; Heath, Fox & Heath 1999; but see Pond & Wu 1981; Fox & Savalli 1998; Mohaghegh, De Clercq & Tirry 1998; Jann & Ward 1999). The most common exceptions to this are cases where females provide maternal care or where the maternal environment triggers major developmental changes in offspring, such as morph differentiation (e.g. winged *vs* non-winged morphs) or the induction of a quiescent stage (e.g. diapause) (Fox & Mousseau 1998). However, the developmental switches affecting these types of traits tend to occur early in development when maternal mRNAs and proteins are most influential inside a cell.

Contrary to the general pattern that maternal effects are generally undetectable in adult offspring is the common observation that offspring from older mothers tend to have shorter adult lifespans. This has been observed in a large diversity of organisms including yeast (Egilmez & Jawinski 1989), plants (Ashby & Wangerman 1954), rotifers (Lansing 1954; but see Verdone-Smith & Enesco 1982), nematodes (Klass 1977), a variety of insects (review in Priest, Mackowiak & Promislow 2002) and even humans (Gavrilov *et al*. 1997) (reviews in Rose 1991; Priest *et al*. 2002). The explanation for this reduction in offspring lifespan

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†Author to whom correspondence should be addressed. E-mail: cfox@uky.edu

with increasing maternal age is unknown, but it may be due to an accumulation of genetic abnormalities in eggs as mothers age (Crow 1997). Alternatively, it may be an artefact of genetic heterogeneity in mortality rates within populations – short-lived females do not contribute offspring to ‘old female’ cohorts, resulting in a population-level shift in the genetic composition of offspring with increasing female age (Vaupel & Yashin 1985). However, some studies have controlled adequately for genetic heterogeneity (e.g. Priest *et al*. 2002) and still detect maternal age effects on offspring lifespan.

With the exception of organisms where fathers provide substantial paternal care, fathers generally provide little more than nuclear genes during fertilization (although in some animals fathers provide materials in their ejaculate that can be incorporated into eggs (review in Vahed 1998), and occasionally paternal mitochondria are incorporated into eggs (review in Birky 2001)). Thus, paternal age generally has little effect on the composition of the egg or the phenotype of his offspring. However, sperm of older fathers may carry more deleterious mutations than sperm of younger males (Crow 1997). In humans, female germ cells undergo ≈24 cell divisions between zygote and egg production whereas male germ cells undergo ≈30 cell divisions prior to puberty and ≈23 cell divisions per year thereafter (Vogel & Motulsky 1997). These extra cell divisions can translate into an increase in the risk of spontaneous mutations (Crow 1999; review in Crow 2000) which could affect offspring longevity. Indeed, offspring of older fathers have been demonstrated to have more congenital problems in humans (reviews in Risch *et al*. 1987; UNSCEAR 2001) and shorter lifespan in a few organisms, including *Drosophila* (Priest *et al*. 2002) and humans (Gavrilov *et al*. 1996, 2000). However, few studies have tried to disentangle the effects of maternal and paternal age (but see Butz & Hayden 1961; Priest *et al*. 2002) or to disentangle the effect of paternal age from the effect of paternal mating history (number of prior mates) on offspring lifespan.

Parental age effects on lifespan can affect the evolution of senescence by altering patterns of age-specific mortality. In general, the magnitude of selection against deleterious traits declines as organisms age, allowing mutations with late-life deleterious effects to accumulate and favouring the evolution of traits with positive effects early in life at the expense of survival and reproduction late in life (Tatar 2001). Parental age effects change the age-specific magnitude of selection on organisms (Kern *et al*. 2001), and can thus change the pattern of accumulation of deleterious alleles and the selective balance for traits that have both early-life and late-life fitness effects (discussed in Priest *et al*. 2002). They can also complicate the analysis of evolutionary experiments that study ageing. For example, evolutionary theories of ageing have been tested using artificial selection experiments in which offspring of older mothers are cultivated for several generations to generate longer-lived strains. Changes in mean lifespan of the selected relative to control lines are interpreted as genetic differences between the lines. The presence of maternal age effects complicate this interpretation by introducing non-genetic differences between the lines and by confounding the source of selection imposed on lines (e.g. confounding natural selection due to increased mortality of offspring produced by older mothers with artificial selection; see Discussion).

We examine the effects of offspring genotype, maternal age, paternal age and paternal mating history on offspring survival, development and adult lifespan in the seed beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae). Maternal effects have been shown for a number of traits in *C. maculatus*; eggs of older mothers are smaller and have lower hatch rate than eggs from younger mothers, and offspring of older mothers have lower egg-to-adult survivorship and take longer to develop to adult (Wasserman & Asami 1985; Fox 1993a; Fox & Dingle 1994; Yanagi & Miyatake 2002). However, none of these studies observed an effect of maternal age on offspring body size at maturation, consistent with the general observation that maternal effects on offspring decline as offspring age and are generally undetectable by the time that offspring mature. We thus have no *a priori* reason to expect maternal age to affect the lifespan of their offspring in *C. maculatus*. However, we find that maternal age does affect offspring lifespan; offspring of older mothers actually live *longer* than offspring of younger mothers, contrary to the pattern observed in many other organisms.

Materials and methods

NATURAL HISTORY AND STUDY POPULATION

Callosobruchus maculatus is a cosmopolitan pest of stored legumes (Fabaceae). Females cement their eggs to the surface of host seeds (Messina 1991). Eggs hatch 4–5 days later (at 26–28 °C) and first instar larvae burrow into the seed. Larval development and pupation are completed within a single seed; beetles emerge as reproductively mature adults.

Having evolved to use dry seeds, and most recently having evolved in a storage environment, *C. maculatus* larvae develop and adults mature, mate and complete reproduction using only metabolic water and the resources acquired during larval development (i.e. they are capital breeders) (Messina & Slade 1997). Access to adult resources has a small positive effect on female fecundity and improves adult lifespan (Fox 1993a,b; Tatar & Carey 1995). However, adults have no access to food or water in a storage environment (they cannot feed externally on seeds) and there is little evidence that they feed as adults outside a storage environment.

All beetles used in these experiments were collected from infested pods of mung bean and the closely related black gram (both *Vigna radiata* L.) in Tirunelveli, India (Messina & Slade 1997), and maintained in laboratory growth chambers on mung bean at >1000 adults per generation for >100 generations, prior to this experiment (the SI or South India population in previous manuscripts).

EXPERIMENTAL DESIGN

We used a traditional half-sibling design to simultaneously quantify genetic variation in larval developmental period and adult lifespan, the genetic correlation between male and female developmental period and lifespan, and the effects of maternal and paternal age on developmental period, lifespan, proportion of eggs that hatched, and egg-to-adult survival. To create half-sib families, virgin male beetles (42 sires) were each mated sequentially to up to five virgin females. Each male, collected within 12 h of his emergence from an isolated host seed, was isolated in a 35-mm Petri dish and allowed to mature for 1 day (males are capable of mating immediately upon emergence but their ejaculate is not fully formed; Fox *et al*. 1995). Each male was then confined in a 35-mm Petri dish with a virgin female that was <12 h post-emergence and allowed to copulate.

To create variation in male age and male mating history, the males were mated on one of two schedules. Twenty-three of the males were mated at 24-h intervals until they fertilized five females, or died. The remaining 19 males were mated sequentially to up to five females in one 3-h period. These two groups of males were thus both mated sequentially to multiple females, but differ in the age at which they mated, allowing us to test for male age and mating order effects on offspring traits. Only males that successfully fertilized at least three females are used in our analyses.

Following mating, all females were transferred to a new 35-mm Petri dish containing 25 mung seeds (their native host) and allowed to lay eggs. Every 12 h the female was transferred to a new dish of seeds. This was continued until each female died or laid eggs for 8 days.

All larvae were reared to adult on mung seeds at densities of one beetle per seed (excess eggs were scraped off), 25 °C, 15L : 9D. Emerging beetles were collected twice daily, at 12-h intervals. All adults were confined individually in a sterile 35-mm Petri dish, at 25 °C, 15L : 9D, and scored twice daily for whether they had died. In total, 8797 offspring were reared to adult and scored for larval developmental period. Because some animals were lost between emergence and death, we measured adult lifespan on 8695 individuals.

ANALYSES

To describe the shape of the mortality curves we estimated the parameter values of a logistic mortality model of the form

$$u(t) = \frac{a\mathrm{e}^{bt}}{\left[1 + \left(\frac{as}{b}\right)\left(\mathrm{e}^{bt} - 1\right)\right]}$$

where a is the intercept of the relationship $u(T) = a\mathrm{e}^{bt}$ (often referred to as the initial mortality rate, or the 'extrinsic' mortality rate), b is the rate of exponential increase in mortality at young ages, and s describes the degree of deceleration in mortality with increasing age (Vaupel 1990; Pletcher 1999a). This is similar to a Gompertz mortality model except that it incorporates a term (s) to account for the slowing of the increase of mortality rate with age (Pletcher, Khazaeli & Curtsinger 2000); when $s = 0$ the logistic model reduces to the Gompertz model. Parameters were estimated using the maximum likelihood estimation procedure of WinModest (Pletcher 1999b). A Cox-proportional hazards model was used to test for overall differences in $u(t)$ (Parmar & Machin 1995; Allison 1995). We used the log-likelihood ratio test of WinModest (Pletcher 1999b) to test whether individual parameter estimates (a, b and s) differed significantly between males and females, and between offspring from different maternal age classes.

Genetic variances for egg-to-adult development time, adult lifespan and total egg-to-death lifespan were calculated using the restricted maximum likelihood variance component estimation procedure of SAS Proc VARCOMP (Littell, Freund & Spector 1991), with V_A (the additive genetic variance) = $4V_S$ (the among sire variance component), and the heritability (h^2) = V_A/V_P (V_P is the total phenotypic variance; Roff 1997). To test for genetic variation in the parameters of the logistic mortality function, we estimated the parameters a, b and s separately for each maternal family (using WinModest; Pletcher 1999b) and then tested for evidence of variation among sires in these parameters using SAS Proc GLM (Littell *et al*. 1991).

Additive genetic correlations (r_A) were calculated both between traits within sexes and between sexes. Between-trait genetic correlations were estimated using standard varcomp procedures in S-Plus (Venables & Ripley 1997; Selvin 1998). Standard errors for r_A estimates were calculated by jackknifing estimates across all sires within each population (Knapp, Bridges & Yang 1989; Fox *et al*. 1999; Windig 1997). Between-sex additive genetic correlations were calculated as $r_A = \sigma^2_{\text{sire-mixed}}/\sigma_{\text{sire-male}}\,\sigma_{\text{sire-female}}$, where $\sigma^2_{\text{sire-mixed}}$ is the estimated sire main effect variance component from the complete mixed model analyses of variance, with progeny sex treated as factor, and $\sigma_{\text{sire-male}}$ and $\sigma_{\text{sire-female}}$ are the estimated sire main effect variance components from the two reduced models (Fry 1992). Data were first standardized to Normal (0,1) to correct for differences in mean and variance between the sexes. Only the sire (co)variance components were used to avoid possible bias due to dominance and maternal effects (Mousseau & Fox 1998). Standard errors for r_A were estimated as

$$SE(r_A) = [(1 - r_A^2)/(2)^{1/2}]\{[SE(h_X^2)SE(h_Y^2)]/(h_X^2 h_Y^2)\}^{1/2},$$

where $SE(h_X^2)$ is the standard error of the heritability estimate of a trait in males and $SE(h_Y^2)$ is the standard error of heritability estimate of a trait in females (Roff 1997).

Results

POPULATION-LEVEL PATTERNS

Averaged across maternal and paternal ages, 97·8 ± (SEM) 0·7% of all eggs hatched, and 93·9 ± 0·8% of the larvae from hatched eggs survived to adult. Females took on average a quarter of a day longer than males to develop from an egg to an adult (egg-to-adult development time pooled across all maternal ages, 27·3 ± 0·1 days for females, 27·0 ± 0·1 days for males). This sex-difference was significant if we included only offspring produced in the first day of maternal egg-laying (sex effect, $F_{1,41} = 6·13$, $P = 0·018$) but not in the analysis of variance pooling all maternal ages (sex effect, $F_{1,41} = 1·93$, $P = 0·17$).

After emergence, females lived on average 22·7 ± 0·3 days and males lived 15·7 ± 0·2 days (averaged across dams within sires, and then across sires; ANOVA sex effect, $F_{1,41} = 115·32$, $P < 0·001$; Fig. 1a). The hazard functions differed substantially between males and females (Cox proportional hazards, $\chi_1^2 = 723$, $P < 0·001$; Breslow ties handling; Fig. 1b) and the hazard functions for males and females were non-proportional (i.e. there was a significant sex × age interaction; $\chi_1^2 = 219$, $P < 0·001$). When the data were fitted to the logistic mortality model all three model parameters differed significantly between the sexes; the intercept was higher for females (suggesting a higher baseline mortality rate; females, $a = 0·00038$; males, $a = 0·00011$; log-likelihood ratio test, $\chi_1^2 = 30·6$, $P < 0·001$) but the mortality rate increased more slowly in females (females, $b = 0·279$; males, $b = 0·577$; $\chi_1^2 = 30·6$, $P < 0·001$), and there was a lower rate of slowing of the increase in the mortality rate in females (females, $s = 0·797$; males, $s = 1·990$; $\chi_1^2 = 30·6$, $P < 0·001$) (WinModest, Pletcher 1999b). In contrast to the results of Fox *et al.* (2003) on this same SI population, but consistent with previous studies of a different population of *C. maculatus* (Tatar & Carey 1994) females showed a delay (≈3 days), relative to males, in the age at which mortality became age-dependent (Fig. 1c).

PATERNAL AGE AND PATERNAL MATING ORDER EFFECTS

After controlling for variation among sires and removing maternal age effects, there were generally no detectable effects of paternal age or paternal mating history (mating order) on offspring phenotypes – although an analysis of variance suggested some heterogeneity among male age classes and across mates when these variables were treated as fixed effects, the effects were undetectable when treated as covariates (which tests for directional effects) (Fig. 2). The one exception was a statistical trend toward male offspring of later matings having shorter adult lifespan. However, this effect was not evident in a graphical analysis of the data (e.g. Fig. 2d), and was not statistically significant after correcting for multiple comparisons. We thus conclude there were no consistent effects of either paternal age or paternal mating history on offspring development time or adult lifespan in *C. maculatus*. Paternal age effects are thus not considered in subsequent analyses.

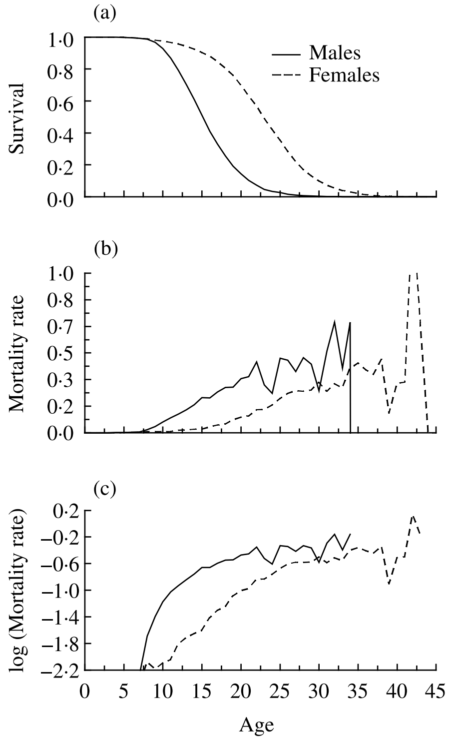

Fig. 1. (a) Survivorship, (b) mortality curve [$u(t)$] and (c) log-transformed mortality curve, $\log[u(t)]$, for male and female *Callosobruchus maculatus*. The mortality curve was best explained by the logistic mortality model, $u(t) = ae^{bt}/[1 + (as/b)(e^{bt} - 1)]$, where a is the initial mortality rate, b is the rate of exponential increase in mortality at young ages, and s describes the degree of deceleration in mortality with increasing age; females: $a = 0·00038$, $b = 0·279$, and $s = 0·797$; males $a = 0·00011$, $b = 0·577$, and $s = 1·990$).

MATERNAL AGE EFFECTS

Eggs laid by older mothers were less likely to hatch (Fig. 3; logistic repeated measures regression; $\chi_{(14)}^2 = 49·9$, $P < 0·001$) and offspring hatching from these eggs had lower egg-to-adult survivorship than offspring hatching from eggs laid by younger females (although the effect was marginally non-significant, $\chi_{(14)}^2 = 22·1$, $P = 0·07$).

Of those that survived to adult, offspring of older mothers took significantly longer to reach maturity and emerge from their host seed than did offspring of

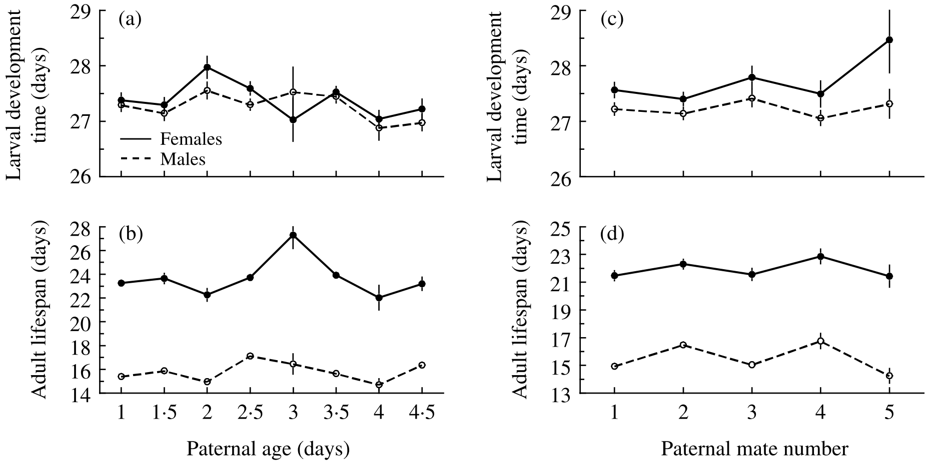

Fig. 2. The effect of paternal age (a, b) and paternal mating history (c, d) on larval development time and adult longevity of male and female *Callosobruchus maculatus*.

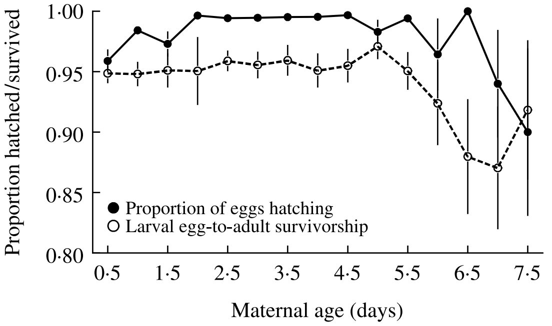

Fig. 3. The effect of maternal age on the on the proportion of her eggs that hatched and the egg-to-adult survivorship of her offspring.

younger mothers (Fig. 4a; analysis of covariance, maternal age treated as a covariate nested within dams; female offspring, $F_{(158,3995)} = 8{\cdot}31$, $P < 0{\cdot}001$; male offspring, $F_{(160,4116)} = 6{\cdot}62$, $P < 0{\cdot}001$). Female development time increased by 0·32 ± 0·05 days for each day of increasing maternal age (slope for regression of development time *vs* maternal age, estimated separately for each dam family and then averaged across all families; $t = 6{\cdot}05$, $P < 0{\cdot}001$), and male development time increased by 0·36 ± 0·04 days for each day of increasing maternal age ($t = 9{\cdot}43$, $P < 0{\cdot}001$). These slopes did not differ significantly between males and females (maternal age × offspring sex interaction, $F_{1,41} = 0{\cdot}01$, $P = 0{\cdot}95$) but did differ significantly among dam families ($F_{117,3995} = 2{\cdot}85$, $P < 0{\cdot}001$).

Contrary to results for *Drosophila* and many other organisms, offspring of older mothers actually lived slightly longer (i.e. had longer adult lifespan) than did offspring of younger mothers (Fig. 4b; effect of maternal age treated as a covariate nested with dams; $F_{(162,8174)} = 1{\cdot}21$, $P = 0{\cdot}03$). However, the slope of the maternal age effect differed significantly between male and female offspring (sex × maternal age interaction; $F_{(161,8174)} = 2{\cdot}50$, $P = 0{\cdot}001$; Fig. 4b). In separate analyses for each sex, this maternal age effect on adult lifespan was statistically significant for male offspring ($F_{161,4058} = 1{\cdot}24$, $P = 0{\cdot}02$) but not for female offspring ($F_{159,3959} = 1{\cdot}08$, $P = 0{\cdot}23$; Fig. 3b). This effect of maternal age on lifespan of male offspring is not an artefact of biased sampling of offspring of older females (e.g. only long-lived females produced offspring at old age, and these offspring could bias upwards the observed lifespan for old maternal ages). Instead, the effect of maternal

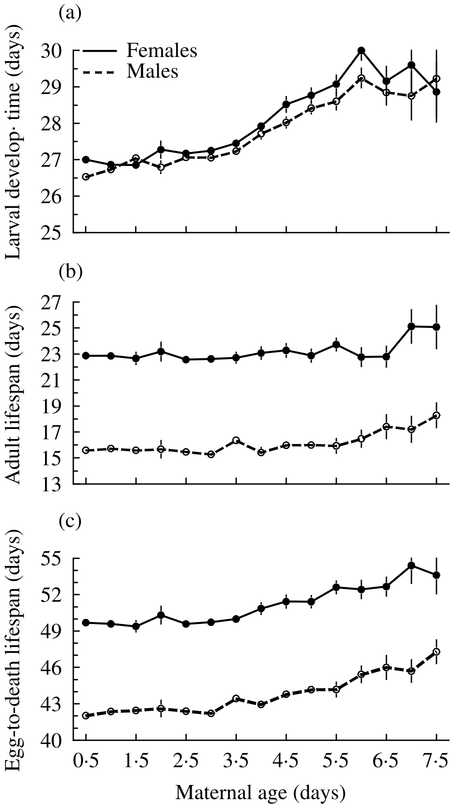

Fig. 4. The effect of maternal age on the phenotype of her offspring; (a) development time, (b) adult (post-emergence) lifespan and (c) total egg-to-death lifespan.

age was significant *nested within dam families*. On average, male adult lifespan increased by 0·15 days for each day of increasing maternal age. The magnitude of the maternal age effect varied significantly among dam families ($F_{120,8334} = 1{\cdot}25$, $P = 0{\cdot}03$).

The shape of the mortality curves varied significantly across maternal age for male offspring ($\chi^2_1 = 14{\cdot}4$, $P < 0{\cdot}001$; Breslow ties handling) but not for female offspring ($\chi^2_1 = 1{\cdot}9$, $P = 0{\cdot}16$). To examine how the parameters of the mortality curves varied across maternal ages, the data were divided into three age classes, offspring produced by mothers <3 days old (young), mothers 3–5 days old (middle), and mothers >5 days old (old). Smaller age classes would substantially reduce sample sizes for estimating parameters. We solved for parameter values of the logistic mortality function and tested for differences in parameter values between each pair of the three maternal age classes (WinModest, log-likelihood ratio test; separate analysis for each sex; Pletcher 1999b). This analysis suggested that both b (the rate of exponential increase in mortality at young ages) and s (the degree of deceleration in mortality with increasing age) differed between offspring of young and old mothers; b decreased significantly with increasing maternal age ($P < 0{\cdot}05$ for male offspring only), and s increased significantly with increasing maternal age ($P < 0{\cdot}05$ for female offspring only).

GENETIC VARIATION AND GENETIC CORRELATIONS

As observed in previous studies for *C. maculatus* (e.g. Fox 1994; Fox *et al.* 2003) the heritability of development time was very low (all $h^2 < 0{\cdot}21$ after removing the effects of maternal age, $h^2 = 0$ if maternal age effects were not removed; $P > 0{\cdot}05$ for all estimates).

The shapes of mortality curves differed significantly among sire families, and among dam families (nested within sires), indicating genetic variation in the shapes of mortality curves (SAS Proc Lifereg: $\chi^2 > 273$, $P < 0{\cdot}001$ for each). To test for variation in the specific parameters a, b and s, we fitted the logistic mortality curve to each full-sib family separately and then used analysis of variance to test for variation in parameter values among sires. Unfortunately, this analysis had little power to detect variation in parameter values owing to limited family sizes within each sire (generally <50 offspring per sex). Thus, we could not detect genetic variance in the parameters (a, b and s) of the logistic mortality function ($P > 0{\cdot}15$ for both the sire and dam effects for all three parameters), although a large sex effect was evident, as in our prior analyses ($P < 0{\cdot}008$ for a, b and s).

The heritability estimates for adult lifespan of both male and female offspring were significantly greater than 0 (Table 1). These estimates differed substantially between the sexes – h^2 was higher for male lifespan than for female lifespan (Table 1). This was due to a substantial increase in the total environmental variance (V_E) for lifespan of females relative to males; the two sexes did not differ for the amount of additive genetic variance (V_A) in lifespan. This same pattern was observed across all maternal age classes. Although these three age classes represent data from the same families, they represent independent estimates of V_A, V_E and h^2 for these families because no offspring are included in more than one maternal age class. The inflated V_E of females relative to males is not an artefact of scale differences between the sexes (females have longer average lifespan); the estimates of the coefficient of environmental variation (CV_E), which correct for scale differences, were approximately twice as high for females as for males.

To test for sire effects on the slope of the relationship between maternal age and both offspring egg-to-adult development time and adult lifespan we estimated the slope of the linear relationship between maternal age and the offspring traits separately for each dam family and then tested for variation in the slopes among sires using analysis of variance. The slope varied among sires for adult lifespan of both male ($R^2 = 0{\cdot}34$, $P = 0{\cdot}03$) and female offspring ($R^2 = 0{\cdot}31$, $P = 0{\cdot}10$), indicating that contributions from males mediate the effect of maternal age on offspring lifespan. For development time, the slope did not vary significantly among sires ($P > 0{\cdot}6$ for both male and female offspring).

Adult lifespan of males was positively genetically correlated with the adult lifespan of females; i.e. sires that produced long-lived sons also produced long-lived daughters, and vice versa (additive genetic correlation, $r_A = 0{\cdot}70 \pm 0{\cdot}13$). Egg-to-adult development time of offspring was not genetically correlated across the sexes, nor was it genetically correlated with adult lifespan for either sex.

Discussion

Consistent with other studies on *C. maculatus* (Wasserman & Asami 1985; Fox 1993a; Fox & Dingle 1994) and a variety of other animals (e.g. Mohaghegh *et al.* 1998; Jann & Ward 1999; Hercus & Hoffmann 2000; Kern *et al.* 2001), we found eggs laid by old mothers were less likely to hatch, larvae hatching from these eggs were less likely to survive to adult and took longer to develop to adult. However, contrary to the results of previous studies on a variety of animals (Ashby & Wangerman 1954; Lansing 1954; Klass 1977; Egilmez & Jawinski 1989; Gavrilov *et al.* 1997; review in Rose 1991; Priest *et al.* 2002), we did not find that offspring of older mothers have shorter lifespans, but that they tend to live longer than offspring of younger mothers. This effect was very small, and statistically significant only for male offspring (although the trend was similar for both male and female offspring). The observed increase in offspring lifespan is not an artefact of genetic heterogeneity within this population (Vaupel & Yashin 1985); we specifically tested for a change in

Table 1. Phenotypic, genetic and environmental variances in adult lifespan (lifespan post-emergence from the host seed) and total egg-to-adult lifespan†

	Variance component			
	Additive genetic variance (V_A)	Environmental variance (V_E)	Total phenotypic variance (V_P)	Narrow-sense heritability (h^2)
Adult lifespan				
Female offspring				
Combined offspring, all maternal ages	7·39 ± 0·85	27·46	34·85	0·212 ± 0·095
Maternal age 0–3 days	8·34 ± 1·00	25·67	34·02	0·245 ± 0·113
Maternal age 3·5–5 days	8·15 ± 1·14	28·04	36·20	0·225 ± 0·123
Maternal age >5 days	0*	33·81	33·81	0*
Male offspring				
Combined offspring, all maternal ages	6·72 ± 0·54	10·85	17·57	0·382 ± 0·114
Maternal age 0–3 days	7·38 ± 0·60	9·70	17·08	0·432 ± 0·127
Maternal age 3·5–5 days	8·02 ± 0·68	9·44	17·46	0·459 ± 0·142
Maternal age >5 days	7·02 ± 1·65	16·21	23·24	0·302 ± 0·277
Total egg-to-death lifespan				
Female offspring				
Combined offspring, all maternal ages	2·79 ± 0·93	49·74	52·53	0·053 ± 0·070
Maternal age 0–3 days	7·55 ± 0·94	28·99	36·53	0·207 ± 0·100
Maternal age 3·5–5 days	7·04 ± 1·11	31·79	38·84	0·181 ± 0·112
Maternal age >5 days	0*	37·62	37·62	0*
Male offspring				
Combined offspring, all maternal ages	4·57 ± 0·74	33·31	37·88	0·121 ± 0·077
Maternal age 0–3 days	8·17 ± 0·65	13·20	21·37	0·382 ± 0·112
Maternal age 3·5–5 days	6·57 ± 0·67	15·85	22·41	0·293 ± 0·114
Maternal age >5 days	12·27 ± 2·08	16·36	28·63	0·428 ± 0·276

Genetic variances were calculated using the restricted maximum likelihood variance component estimation procedure of SAS Proc VARCOMP (Littell *et al*. 1991). Standard errors for h^2 calculated following Becker (1992). See Fox (1994), Fox (1998) and Fox *et al*. (1999) for other examples of these procedures.
*Estimated among-sire variance component was 0, resulting in a 0 V_A and 0 h^2.
†Data are not presented for egg-to-adult development time because no V_A or h^2 estimates different significantly from 0.

offspring lifespan *within* families, thus removing the confounding effects of genetic heterogeneity among families. The increase in offspring lifespan with increasing maternal age is also not a consequence of a maternal age effect on offspring body size; we have no evidence in *C. maculatus* that offspring body size increases with increasing maternal age (Wasserman & Asami 1985; Fox 1993a; Fox & Dingle 1994), and even if there was a small positive effect of maternal age on offspring size, the relationship between body size and lifespan is at best very weak in this *C. maculatus* population (Fox *et al*. 2003).

Few studies have tried to disentangle the effects of maternal and paternal age (but see Butz & Hayden 1961; Priest *et al*. 2002) or to disentangle the effect of paternal age from the effect of paternal mating history (number of prior mates) on offspring growth or life history. In our study, maternal age was not confounded with paternal age at the time of mating. We can thus conclude that the age at which a male fertilizes a female does not affect the lifespan of her offspring. However, females were mated only once and the sperm used to fertilize later-produced offspring were stored in the spermatheca and aged as the female aged. We thus cannot disentangle effects of female age from sperm age. Sperm mobility and the ability of sperm to fertilize ova decrease with sperm age (e.g. Powell, Tyler & Peck 2001) and the genotypes of sperm that fertilize eggs differ across time (Sapp & Martindeleon 1992), with less functional sperm (which often carry genetic disorders) more likely to fertilize ova later (Aranha & Martindeleon 1995). Also, DNA may deteriorate and thus sperm may accumulate genetic damage as they age (Siva-Jothy 2000). Thus, ageing of sperm may explain why egg hatch and offspring egg-to-adult survivorship both decrease with increasing maternal age. However, these mechanisms would predict a decline in offspring lifespan with increasing maternal/sperm age, contrary to the result of our study.

As females age, they may shift which mRNAs and proteins are packaged into eggs by nurse cells, affecting the development and life history of their offspring (including lifespan). Alternatively, the observed increase in offspring adult lifespan with increasing maternal age may be due to differential mortality of genotypes *within* families. It is clear from Fig. 3 that egg hatch and offspring egg-to-adult survivorship begin declining after mothers pass age 5 or 5·5 days. This is the same maternal age after which an increase in offspring adult lifespan becomes observable (Fig. 4b). We suggest that these may in part reflect a cause–effect relationship. Eggs laid by older mothers are substantially smaller than eggs laid by younger mothers (Wasserman & Asami 1985; Fox 1993a; Yanagi & Miyatake 2002) and are at greater risk of mortality. If mortality differentially affects offspring according to their general vigour, then the increased mortality at older maternal ages could remove lower-quality individuals from the sample of offspring produced by older mothers. Offspring successfully developing to adult from eggs laid by older mothers would thus be a non-random sample of all the offspring produced by those mothers (only the most vigorous offspring are left) which, if larval vigour is correlated with adult vigour, would live longer as adults than would a sample of offspring that had not been subjected to selection as larvae. Thus, although the observed pattern is not a result of genetic heterogeneity among families, it may be the result of genetic heterogeneity *within* families and natural selection on offspring vigour during development, the effects of which carry over to affect adult lifespan.

The effect of maternal age on offspring lifespan differed between male and female offspring; although the trend of longer adult lifespan for offspring of older mothers was similar in both sexes (Fig. 4b), the maternal age effect was statistically significant only for male offspring and the average slope of the maternal age effect differed significantly between male and female offspring. Previous studies have likewise found that the effect of maternal age on offspring lifespan differs between male and female offspring (e.g. Gavrilov *et al*. 1997), although which sex shows the greater effect varies among studies and even among populations within studies (Priest *et al*. 2002). This sex difference may result from sex differences in the expression of genes that affect lifespan. Sex differences in gene expression are common (Rice & Chippindale 2001; Nuzhdin *et al*. 1997; Nuzhdin & Reiwitch 2002) and consistent with the generally low genetic correlation observed between male and female lifespan in *C. maculatus* ($r_A = 0{\cdot}70$ in this study; $r_A = 0{\cdot}78$ and 0·84 in two other studies; Fox *et al*. 2003; these estimates of r_A are substantially lower than the between-gender genetic correlations for body size and many other traits; Czesak & Fox 2003a,b). Some genes affecting longevity may be on the sex chromosomes, although there are few demonstrations of this (Woodruff 1992).

In this study, the magnitude of the maternal age effect on offspring lifespan varied among sire families. Assuming there are no effects on lifespan inherited non-genetically through fathers (an assumption consistent with our results), this result suggests that the degree to which offspring are sensitive to maternal effects depends on offspring genotype. Numerous studies have demonstrated genetic variation in the degree to which mothers influence the phenotype of their offspring (demonstrating genetic variation in the maternal effect; e.g. Byers, Platenkamp & Shaw 1997; Fox *et al*. 1999), and some studies have shown that the degree to which offspring respond to a maternal effect depends on offspring genotype (e.g. Byers *et al*. 1997; Wolf 2000; Evans & Kermicle 2001). However, we know of no previous studies demonstrating that the magnitude of the maternal age effect experienced by offspring depends on offspring genotype. Alternatively, this result may reflect a complex interaction between female genotype and the genotype of the sperm that fertilize her eggs. For example, in *Drosophila melanogaster*, the genotype of sperm that can successfully fertilize a female's eggs depends on the genotype of the female producing the eggs (Clark, Begun & Prout 1999), creating a male genotype × female genotype interaction effect on offspring phenotype. If this interaction between sperm changes as female's age, it could produce a maternal age × sire interaction, as observed. These hypotheses cannot be distinguished with our data.

We have examined only one population of *C. maculatus* and thus cannot generalize from our study to other populations of *C. maculatus*. It is likely that the relative influence of maternal age on offspring survival varies among populations, as has been demonstrated for *Drosophila*. Priest *et al*. (2002) found that lifespan increased with maternal age in one outbred population of *D. melanogaster* but decreased with maternal age in other populations. Populations of *C. maculatus* have diverged substantially in a whole suite of morphological and life-history traits, including egg size, body size, paternal investment, oviposition preference, egg dispersion and patterns of adult mortality (Fox *et al*. 2003; Messina & Slade 1997; Savalli *et al*. 2000). We are currently examining the degree to which maternal effects on a variety of these traits vary within and among populations.

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