Is a decline in offspring quality a necessary consequence of maternal age?

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Recent studies in two species of *Drosophila* have demonstrated a negative effect of parental age on offspring fitness, including a reduced hatch rate of eggs and larval-to-adult viability. This has led to a call to consider the decline of offspring quality as a function of parental age in theoretical considerations of the evolution of ageing. We have tested whether a decline in egg and larval quality of older mothers is a general feature of senescence by examining it in the cockroach *Nauphoeta cinerea*. We also tested whether maternal age affected the reproductive potential of daughters. Although maternal age at first reproduction profoundly affected maternal fitness, there was no difference in hatch rate or larval viability between the offspring of young and old mothers. Likewise, the reproductive potential of the daughters of young and old mothers was the same. Thus, while maternal age effects may be important aspects of ageing in some systems, the generality and overall importance for theories of ageing remain unclear.

Keywords: ageing; offspring quality; maternal age effects; senescence

1. INTRODUCTION

The evolution of senescence is typically explained as a consequence of several general processes: accumulation at advanced ages of deleterious alleles that are subject only to weak counter-selection (Medwar 1952); antagonistic pleiotropy, where genes that are beneficial at young ages have detrimental effects at later ages (Williams 1957); and trade-offs between somatic maintenance and repair and investment in growth and reproduction (Kirkwood 2002). These hypotheses have received empirical support, particularly in studies of *Drosophila* (e.g. Rose 1984; Partridge & Fowler 1992; Zwaan *et al.* 1995). Central to the existing senescence theory is the assumption of a purely genetic effect of senescence, that is, that there is no persistent senescent phenotypic effect transferred to offspring (Priest *et al.* 2002). However, negative phenotypic effects of parental senescence on offspring fitness are constrained by neither selection nor theory.

In many taxa, offspring of older mothers often have a reduced lifespan, a phenomenon referred to as the Lansing effect (Lansing 1947; Priest *et al.* 2002). Several studies have also documented a reduction in the viability of

embryos or juveniles associated with parental age. For example, in mammals, the reproductive potential of older females declines with age, primarily owing to a loss of oocytes (Fitzgerald *et al.* 1998). The quality of the remaining oocytes also declines in older females; embryos produced by both *in vivo* and *in vitro* fertilization of oocytes of older females have lower viability (Fitzgerald *et al.* 1998). In addition, recent studies on *Drosophila* species show that maternal age is negatively correlated to egg-hatching rate and that the decrease in offspring quality persists post-embryonically, with a reduction in larval viability for the offspring of old mothers (Hercus & Hoffman 2000; Kern *et al.* 2001).

Age-related effects are attributed to declining parental condition owing to senescence that may affect parental care quantity and quality, or the presence of deleterious factors in the maternal environment during early development. Several recent studies have suggested that such negative parental effects on offspring fitness may be important in the context of the evolution of senescence, as consideration of age-specific parental fitness should include offspring quality (Kern *et al.* 2001; Priest *et al.* 2002). Evidence for effects of parental senescence on offspring quality is rare, although genetic variation for the pattern of offspring quality decline with parental age has been demonstrated in *Drosophila* (Kern *et al.* 2001).

In this study, we investigated parental senescence effects on offspring quality in the cockroach *Nauphoeta cinerea*. Female age is negatively correlated with fertility and lifetime fecundity in this species (Moore & Moore 2001), however, maternal effects on offspring quality have not been studied. The life history of *N. cinerea* is very different from that of *Drosophila*. *Nauphoeta cinerea* is ovoviviparous; although no nutrients are passed from the mother to the developing embryos, females carry the embryos in a brood pouch for approximately five weeks until the offspring emerge as first-instar nymphs (Roth & Willis 1954), thus any negative maternal environment effects are likely to be strong in the species. We compared a number of characters as measures of offspring quality in the first clutch of offspring from young and old females in *N. cinerea*, including egg production, larval-to-adult viability, nymph development rate and the fertility of daughters at different ages. Given the strong effect of maternal age on fertility in *N. cinerea*, and the effects of age on offspring quality in the two *Drosophila* species (Hercus & Hoffman 2000; Kern *et al.* 2001), we predicted that we would find significant differences in offspring quality between the offspring of young and old mothers.

2. MATERIAL AND METHODS

(a) Experimental design

Details of *N. cinerea* husbandry can be found in Moore & Moore (2001). As in that study, females were mated to random males at either 7 (young) or 18 (old) days after adult emergence, which coincide with the two extremes of age effects on fertility. All males used were mated to females at 10 days after adult emergence to control for paternal age effects. Females and offspring were reared in individual containers at 27 °C in an incubator with *ad libitum* food and water.

(b) Effect of maternal age on egg production

In the first experiment, ootheca were removed from the brood pouches of females 28 days after mating. The oocytes in each ootheca were counted and the counts were recorded. At this stage, developing embryos can be distinguished from aborted oocytes. The number of non-developing embryos was noted. Oothecae were imaged on a Leica stereomicroscope fitted with a Nikon coolpix900 digital camera. Embryo images were measured with NIH IMAGE.

Figure 1. Oothecae removed 28 days after mating from either a young or an old mother. The eggs from the old mother are noticeably smaller than those from the young mother. In addition, there are fewer oocytes in the old mother's ootheca.

(c) **Effect of maternal age on maternal fitness and offspring quality**

In the second experiment, mated females were checked daily for parturition of offspring or dropped ootheca. At parturition, the date was recorded and the offspring were counted and removed from their mother and reared in a separate container under standard conditions. The clutches were checked daily for the emergence of the first adult. Nymph development time was compared using the number of days to the emergence of the first adult. On the day of emergence of the first adult the offspring were counted to assess larval survivorship.

To assess the maternal effects on fertility of daughters, two females from each mother were retained for mating. One daughter was mated at a young age (at 7 days post adult emergence) and one daughter was mated at an old age (18 days post adult emergence). All daughters were housed under standard rearing conditions until parturition of their first clutch. The days to parturition were again recorded, as well as the number of offspring.

(d) **Statistical analysis**

We used analysis of variance (ANOVA; Sokal & Rohlf 1995) to examine how offspring number and quality changed with maternal age. All statistical analyses were carried out using SYSTAT v. 9.0.

3. RESULTS

(a) **Age at mating affects female fitness**

As expected from our previous research (Moore & Moore 2001), the age at which a female mates significantly affects the number of offspring produced in her first clutch. Thus, old mothers produced fewer offspring than young mothers ($F_{1,96} = 23.030$, $p < 0.001$).

Oothecae were examined to determine whether the reduction in the offspring number resulted from reduced numbers of oocytes or reduced viability of oocytes (figure 1). Old females had significantly fewer eggs packaged into ootheca than young mothers (figure 2a; $F_{1,17} = 22.94$, $p < 0.001$). Most oocytes appeared to be developing normally; aborted or unfertilized ova were rare regardless of the age of the mother. In addition, embryos in oothecae of old mothers were significantly smaller than those of young mothers even though they were all collected at the same number of days following mating (figure 2b; $F_{1,150} = 10.019$, $p = 0.002$), indicating delayed development.

Figure 2. Age at first reproduction affects both the number of eggs packaged into the ootheca and the development rate of the embryos. (a) Old mothers package significantly fewer oocytes into their oothecae than young mothers (means $\pm$ s.e.; $n_{\text{young}} = 9$ oothecae, $n_{\text{old}} = 10$ oothecae). (b) The embryos of old mothers are less well developed than those of young mothers (means $\pm$ s.e.; $n_{\text{young}} = 72$ embryos, $n_{\text{old}} = 80$ embryos).

(b) **Maternal age at mating has no effect on offspring fitness**

Larval viability was not affected by maternal age ($F_{1,86} = 0.429$, $p = 0.514$). Nymph development rate was also not affected by maternal age. There was no significant difference between young and old mothers in offspring age at first adult emergence ($F_{1,86} = 0.282$, $p = 0.597$).

There was no maternal effect on the fertility of females mated at 7 days of age ($F_{1,81} = 0.082$, $p = 0.775$) or at 18 days of age ($F_{1,78} = 0.085$, $p = 0.772$). The mean number of offspring in the first clutch was 35 for both the daughters of young and of old mothers mated at 7 days of age and 26 for the daughters of young and of old mothers mated at 18 days of age.

Maternal age has a significant effect on the length of gestation, with old mothers taking significantly longer to produce a first clutch than young mothers (Moore & Moore 2001). We found a small maternal effect on development time in daughters mated at 7 days of age ($F_{1,81} = 3.660$, $p = 0.059$). There was no maternal effect on development length between the daughters of young and old females mated at 18 days of age ($F_{1,78} = 0.9491$, $p = 0.333$).

4. DISCUSSION

Theoretical considerations of the evolution of ageing assume that the effects of ageing are confined to a single generation; that is, that the phenotypic expression of age-related changes do not persist to the next generation (Rose 1991; Kern *et al.* 2001; Priest *et al.* 2002). However, a negative effect of maternal age on offspring lifespan has been shown in several taxa (Priest *et al.* 2002 and references therein). Recent studies in *Drosophila* have also demonstrated maternal age effects on larval viability (Hercus & Hoffman 2000; Kern *et al.* 2001). Genetic variation in maternal effects on both adult and juvenile mortality in *Drosophila* suggests that parental effects could play a fundamental part in the evolution of ageing (Kern *et al.* 2001; Priest *et al.* 2002). Accordingly, the authors of these studies have called for the integration of offspring quality into a comprehensive theory of senescence, especially in organisms in which the maternal contribution includes more than the supply of the oocyte cytoplasm.

However, Kern *et al.* (2001) ask the critical question of how commonly a decline in offspring quality accompanies parental ageing. There is some evidence that, in some species other than *Drosophila*, there is no effect of maternal age on the egg-hatching rate and larval survival (McIntyre & Gooding 1998; Mohaghegh *et al.* 1998; Mousseau & Dingle 1991). Our results demonstrate that in the ovoviparous cockroach, *N. cinerea*, there is no effect of maternal age on offspring fitness either as embryos or juveniles. The reproductive biology of *N. cinerea* suggests that maternal age effects should be exacerbated in that the female provides both the oocyte cytoplasm and a brood environment. One potential difference between *Drosophila* and *N. cinerea* may lie in the role of maternal genes and nurse cells during oogenesis between meroistic and panoistic ovaries (Chapman 1998). While it is always difficult to argue negative results, the large sample sizes in our experiment suggest that the biological effect of maternal age on offspring fitness is not significant.

While it is clear that maternal age effects are evolutionarily important and should be further explored theoretically in the context of the evolution of senescence, the results of this study demonstrate the risk of drawing universal conclusions based solely on a single model organism (Bolker & Raff 1997). In addition, the evolutionary implications of the impact of maternal age on adult viability, which appears to be widespread taxonomically, as opposed to juvenile viability, which may be more limited,

need to be considered separately. We agree with Kern *et al.* (2001) and Priest *et al.* (2002) regarding the importance of parental age effects in life-history evolution. There is a need, however, for more studies on the impact of parental age on offspring quality from organisms with diverse life histories and reproductive biology.

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