The Delayed Impact of Parental Age on Offspring Mortality in Mice

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The certitude of death makes reproduction the foundation upon which all life-history strategies are based. Plasticity in the reproductive biology of organisms is an essential adaptive response to the capricious and hazardous environments of earth. In this article, we use data from a breeding colony for laboratory mice to examine the mortality risks of offspring born at the outer boundaries of their Dam’s reproductive plasticity. Our results suggest that the mortality/survival characteristics of offspring are affected by both litter parity and offspring gender. Females born to young Dams have consistently longer life spans than females born to older Dams. Conversely, males are either not affected by parental age or have longer life spans when born to older Dams.

Key Words: Maternal age—Offspring quality—Reproductive senescence—Stochastic effects.

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The biological consequences that arise from reproductive plasticity have been extensively documented in the scientific literature, ranging from the relationship that exists between clutch size and fledgling survival (1,2) to the demonstration that restricting egg laying to older and still fecund Drosophila melanogaster increases the longevity of subsequent generations (3). Selection, however, typically involves trade-offs (4), and selection for extended longevity is no exception; extended longevity in Drosophila is accompanied by reduced developmental viability and increased developmental lethality (5). At the other end of the spectrum, Snowdon and colleagues (6) suggested that early menopause in humans is a biomarker of increased mortality risks for that individual.

Studies have now confirmed the trade-off between longevity (maintenance of the soma) and reproduction (7–11) predicted by the disposable soma theory of ageing (12,13). Greater investment in reproduction (more offspring and/or earlier age at first reproduction) shortens longevity (14–19), whereas fewer offspring, reduced offspring fitness, and/or delayed age at first reproduction are positively correlated with greater longevity (3,20,21). Senescence, an unintended by-product of the biology responsible for the longevity needed to achieve Darwinian fitness (22), is presumably either delayed or accelerated by extending or truncating the timeframe (from conception to the effective end of reproduction) over which selection operates (23).

Less is known about how parental age affects the health and longevity of offspring. Already in 1946, Pearl (24) was one of the first scientists to comment on this issue when his analysis of human pedigree data led him to conclude that in order to be longevous, one should “pick long-lived parents, and particularly long-lived mothers.” Evolutionary theories of ageing, on the other hand, tend to focus on the biological consequences affecting the individual (the soma) rather than their offspring (3,13). The last decade, however, has produced a multitude of studies that found evidence for negative effects on offspring fitness associated with parental age (eg, (25–29)), sometimes even including effects spanning three generations from grandparents to current offspring (30,31). Understanding life-history evolution and population dynamics, therefore, requires a detailed understanding of how an organism’s fitness (ie, reproduction and survival) varies not only with the age of that individual but also with the age of its parents (27,32,33).

Numerous studies on wild vertebrates demonstrate that middle-aged parents usually rear more offspring than young or old parents (11,34,35). Life-history theory, however, predicts a trade-off between offspring number and offspring size and/or quality (4). Thus, fitness cannot be measured simply by the number of offspring reared to independence. Although number of offspring reared is an important component of fitness, we think that the negative effects of parental age experienced during development can have consequences that persist well beyond the age of independence (30). As such, we suggest that overall offspring survival, regardless of clutch size, is a more appropriate measure of individual fitness for offspring and parents (see also discussion in (21,27)).
Several recent studies on laboratory-reared animals (26, 27, 36) and wild populations (21) have successfully demonstrated that offspring viability and/or life span are indeed negatively affected by parental age. This effect is not restricted to nonhuman animals. Using human genealogical data, Gavrilo and Gavrilo (37) reported that daughters but not sons of older fathers lived shorter lives; unfortunately, sampling statistics precluded a comparable analysis of maternal age. Conversely, in 2000, Gudmundsson (38) reported that sons of long-lived fathers as well as sons and daughters of long-lived mothers had significant reductions in mortality relative to the offspring of less long-lived parents. Clearly, there seems to be a strong influence of parental age at reproduction on the survival characteristics of offspring.

The logic motivating this article is based, therefore, on the plausible assumption that reproductive senescence and the physiological costs associated with iteroparous reproduction impose an accumulating stress on Dams (mothers) that, in combination, will affect the subsequent mortality characteristics of their offspring. If true, the mortality characteristics of offspring should depend upon their position on what is effectively a physiological stress continuum for their mothers. A simple test of this assumption will be presented that compares mortality characteristics of offspring born at opposite ends of the reproductive window (ie, offspring born to young Dams with few parities vs offspring born to old Dams with numerous parities).

**Materials and Methods**

**Study Animals**

A breeding colony of laboratory mice was maintained in the Division of Biological and Medical Research at Argonne National Laboratory from 1953 to 1969 in order to provide laboratory mice for experimental studies (39). Data for this colony included 3,875 Dams distributed across 39 mouse strains (4 inbred strains, 4 hybrid strains, 11 backcross strains, 4 outcross/backcross strains, and 16 inbred and marker stock strains brought from Iowa State to Argonne National Laboratory in 1963).

We selected four inbred strains from that colony for this study: A-Strain, BALB/c, C3HF and C57BL. Each Dam was individually caged with a male from the same litter (ie, full sib). The number of inbreeding generations in these strains ranged from 20 to 125. Electronic records exist for every Dam; these records include Dam and Sire (father) ID, dam and sire birth date, each mating date, number and sex of pups born, preweaning death count organized into three age intervals (0–5 days, 6–15 days, and 16 days to weaning), number of pups that survived to weaning, eventual discard date for either Dam or sire, and the reason for discard.

**Offspring Selection**

The 580 Dams produced 2,406 control offspring. Mice born to Dams with fewer than four parities and an age at birth of litter that fell within the lowest 1/3 of the birth age distribution (N = 691) were considered offspring of “likely unstressed” young Dams (designated US). Conversely, mice born to Dams with more than three parities and an age at birth of litter that fell within the upper 1/3 of the birth age distribution imposed an accumulating stress on Dams (mothers) that might produce offspring more like those born to older Dams, which could bias the offspring analyses. A comparison of mean litter size and proportion of pups weaned within the first two parities revealed that mean litter size was unaffected (Table 1), but Dams with less than three parities weaned a significantly smaller proportion of their pups (Figure 1). As a result, the 1,580 Dams were reduced to 1,385 and only 580 of those produced offspring assigned to the control groups (Table 2).

**Table 1. Analysis Used to Exclude Dams and Their Offspring Who Would Have Otherwise Been Included in Subsequent Analyses**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mean Litter Size (SE)</th>
<th>Proportion Weaned</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;3 Parities</td>
<td>&gt;2 Parities</td>
</tr>
<tr>
<td>A</td>
<td>5.2 (0.25)</td>
<td>5.3 (0.06)</td>
</tr>
<tr>
<td>BALB/c</td>
<td>5.2 (0.41)</td>
<td>4.9 (0.11)</td>
</tr>
<tr>
<td>C3HF</td>
<td>5.8 (0.32)</td>
<td>6.3 (0.09)</td>
</tr>
<tr>
<td>C57BL</td>
<td>5.3 (0.54)</td>
<td>6.7 (0.09)</td>
</tr>
</tbody>
</table>
distribution ($N = 628$) were considered offspring of “likely stressed” older Dams (designated LS). Mice falling between these two extremes or mice assigned to an aging study with a delayed age of entry or reassigned to be breeders or transferred to other experiments or selected for a planned sacrifice were excluded ($N = 1,087$). This division of maternal age into tertiles utilizes the minimum number of partitions required to compare the extremes (ie, young vs old) while also maximizing the sampling statistics for the analyses used in those contrasts. Table 3 provides summary information for the 1,319 mice selected for analysis. Although these animals were maintained under the best husbandry practices available at the time, they lived out their lives with no veterinary interventions.

Methods of Analysis

Three distinct but related methods of analysis were used to formally test whether survival differences exist between the US and LS groups; one method uses a nonparametric approach and the other two methods are parametric. Kaplan–Meier analysis is a well-known and widely used nonparametric procedure that provides two tests for homogeneity among two or more cumulative survivorship curves (one statistic sensitive to differences at early ages, Wilcoxon, and another statistic sensitive to differences at older ages, logrank).

The two parametric approaches fit either a Weibull or Gompertz model to the failure time data. Parametric models are useful because they permit age patterns of mortality to be viewed as cumulative survivorship curves like the Kaplan–Meier analysis or as density functions or hazard functions. The latter function is particularly informative because it provides insight into age-specific changes in mortality rather than the cumulative changes described by Kaplan–Meier. In combination, the Weibull and Gompertz models have played a dominant role in the analysis of failure time data (40).

All three approaches allow for what is called competing risks (ie, multiple causes competing for the lives of individuals) where the causes of interest are events and the other causes of death or loss to follow-up are called censored observations. This distinction is important because it allows for biologically motivated partitions of mortality to be employed (41). Specifically, in this analysis, intrinsic events (natural deaths) are distinguished from extrinsic events (eg, premature deaths or losses like escapes or animal husbandry accidents). Hence, intrinsic events are the focus of this analysis because they reflect the mortality that arises from the “intrinsic” biology of the mouse strain. Both types of events contribute to the analysis and impact the ultimate estimation of model parameters. In other words, censoring is not the same as deleting censored events from an analysis. It should be noted that only 1.5% ($N = 20$) of the 1,319 deaths were censored.

Results

Despite using different statistics, the results from the three methods of analysis used in this study (Table 4) and their visual representation (Figure 2) lead to a convergence of interpretation. First and foremost, the basic premise that maternal age at birth of litter has an impact on the mortality attributes of offspring was confirmed. With one exception (the C57BL strain), the $p$ values from all three methods were either significant ($\leq .05$) or clearly suggestive ($<.20$). In other words, the offspring of younger mothers have a different age pattern of mortality risks than those of older mothers. The second most salient feature of these results is the finding that the mortality consequences of maternal age for offspring also depend on their gender. Female offspring born to older mothers have consistently lower survival than their counterparts born to young mothers (Figure 2). Visually, the male results lack the consistency of trend observed in the female data. Statistically, the results suggest that maternal age at birth of litter either has no adverse affect on the mortality/survival attributes of male offspring (C57BL...
and BALB/c) or being born to older mothers actually improves those attributes (A, C3Hf).

The question most likely to arise when reading these results is why was there no maternal age effect on either female or male C57BL offspring? There is no definitive answer to this question, but there are historical clues (conversation with Dr. Douglas Grahn, see “Acknowledgments”). As described in the “Materials and Methods,” these mice were used in radiation studies conducted between 1953 and 1970, the so-called Pre-JANUS period. Once the JANUS biomedical reactor (1970–1992) was built at Argonne National Laboratory, the decision was made to create a hybrid mouse (with the anticipated hybrid vigor) that would become the standard mouse for all subsequent studies. From these results, it is easy to guess one of the strains used in creating that hybrid, the C57BL strain. The hybrid was designated as B6CF1 and arose from the cross between a female C57BL and a male BALB/c. The C57BL was chosen because of its excellent life expectancy (relative to other strains, see Figure 2), an attribute based upon resistance to both infectious and neoplastic disease—important traits when studying radiation-induced cancer. The BALB/c was chosen not for its robustness but because it was a well-known and widely used mouse strain that also was not susceptible to cancers. Thus, both female and male C57BL mice are exceedingly robust and, by inference, would be more resistant to the consequences of the physiological stress continuum hypothesized in this study.

**DISCUSSION**

As we discuss the implications of these analyses, it is important to put them into their proper context. The objective of scientific breeding colonies is to eliminate genetic variation via inbreeding in order to produce animals that are as close to clones as possible (42,43). The perceived success of achieving this goal has, in fact, been used to argue that laboratory animals are not good models for understanding biological processes in humans (eg, (43,44)). To carry this logic to its demographic extreme, the survival curve of inbred mouse strains should be square (ie, the mice all die at the same time) or at a minimum, the deaths should be compressed into a narrow age range.

The biological reality, however, is much different; the survivorship curves of inbred mice look much like those of hybrid mice (45) or even feral mice (Figure 3), and the survivorship curves of laboratory animals such as dogs and mice look like those for humans when compared on a biologically comparable time scale (46–48). If only a small fraction of the distributed deaths of inbred animals can be attributed to genetic variation (43), then the next likely culprit would be environmental factors and/or gene–environment interactions. However, controlled laboratory settings are specifically designed to minimize those sources of variation (see (49) for a counter example of between-laboratory effects). By a process of elimination, the remaining source of variation responsible for this unexpected heterogeneity must involve effects that are postconception (eg, epigenetic, senescence, wear, and tear) and the evidence suggests that these effects may be significant contributors to the ultimate demise of individuals (eg, (50,51)).

### Table 3. Summary Information for the 1,319 Offspring Used to Test for the Effect of Maternal Age (expressed as days); Mean and Standard Error for Age of Dam at Birth of Litter, Number of Parities, Age At Entry Into Control Population, and Age At Death

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>Dam Group</th>
<th>N</th>
<th>Dam Age (Mean ± SE)</th>
<th>Parity (Mean ± SE)</th>
<th>Entry Age (Mean ± SE)</th>
<th>Death Age (Mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>M</td>
<td>US</td>
<td>130</td>
<td>98 (1.4)</td>
<td>1.4 (0.1)</td>
<td>111 (6.3)</td>
<td>569 (15.0)</td>
</tr>
<tr>
<td>BALB/c</td>
<td>US</td>
<td>66</td>
<td>262 (4.4)</td>
<td>6.7 (0.1)</td>
<td>113 (9.0)</td>
<td>615 (19.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LS</td>
<td>69</td>
<td>116 (2.8)</td>
<td>1.6 (0.1)</td>
<td>86 (3.1)</td>
<td>490 (24.2)</td>
<td></td>
</tr>
<tr>
<td>C3Hf</td>
<td>US</td>
<td>61</td>
<td>327 (4.1)</td>
<td>6.9 (0.2)</td>
<td>90 (1.8)</td>
<td>543 (24.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LS</td>
<td>80</td>
<td>95 (1.7)</td>
<td>1.3 (0.1)</td>
<td>111 (11.6)</td>
<td>640 (29.7)</td>
<td></td>
</tr>
<tr>
<td>C57BL</td>
<td>US</td>
<td>93</td>
<td>266 (4.8)</td>
<td>6.7 (0.2)</td>
<td>108 (7.4)</td>
<td>619 (19.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LS</td>
<td>70</td>
<td>116 (2.0)</td>
<td>1.6 (0.1)</td>
<td>112 (6.5)</td>
<td>687 (19.7)</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>F</td>
<td>US</td>
<td>137</td>
<td>98 (1.3)</td>
<td>1.3 (0.1)</td>
<td>108 (5.4)</td>
<td>638 (13.3)</td>
</tr>
<tr>
<td>BALB/c</td>
<td>US</td>
<td>82</td>
<td>252 (2.2)</td>
<td>5.9 (0.1)</td>
<td>125 (5.4)</td>
<td>617 (13.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LS</td>
<td>73</td>
<td>126 (2.4)</td>
<td>2.0 (0.1)</td>
<td>96 (2.6)</td>
<td>565 (19.9)</td>
<td></td>
</tr>
<tr>
<td>C3Hf</td>
<td>US</td>
<td>65</td>
<td>325 (4.6)</td>
<td>7.3 (0.2)</td>
<td>92 (2.3)</td>
<td>581 (18.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LS</td>
<td>86</td>
<td>110 (2.8)</td>
<td>1.5 (0.1)</td>
<td>99 (5.4)</td>
<td>733 (25.8)</td>
<td></td>
</tr>
<tr>
<td>C57BL</td>
<td>US</td>
<td>62</td>
<td>291 (3.6)</td>
<td>6.6 (0.2)</td>
<td>97 (3.7)</td>
<td>725 (18.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LS</td>
<td>83</td>
<td>130 (4.4)</td>
<td>6.7 (0.1)</td>
<td>113 (9.0)</td>
<td>615 (19.6)</td>
<td></td>
</tr>
</tbody>
</table>

Note: K-M = Kaplan–Meier; LS = “likely stressed” older Dams; US = “likely unstressed” young Dams

### Table 4. Analysis Summary of p Values for K-M (Wilcoxon/ logrank), Weibull and Gompertz Contrast of US Versus LS

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sex</th>
<th>K-M</th>
<th>Weibull</th>
<th>Gompertz</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>F</td>
<td>0.1440.01</td>
<td>0.56</td>
<td>0.096</td>
</tr>
<tr>
<td>BALB/c</td>
<td>M</td>
<td>0.05/0.22</td>
<td>0.167</td>
<td>0.004</td>
</tr>
<tr>
<td>C3Hf</td>
<td>M</td>
<td>0.06/0.08</td>
<td>0.074</td>
<td>0.010</td>
</tr>
<tr>
<td>C57BL</td>
<td>M</td>
<td>0.07/0.03</td>
<td>0.111</td>
<td>0.172</td>
</tr>
<tr>
<td>A</td>
<td>F</td>
<td>0.00/0.00</td>
<td>0.004</td>
<td>0.145</td>
</tr>
<tr>
<td>C3Hf</td>
<td>M</td>
<td>0.28/0.21</td>
<td>0.176</td>
<td>0.164</td>
</tr>
<tr>
<td>C57BL</td>
<td>F</td>
<td>0.50/0.34</td>
<td>0.493</td>
<td>0.196</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>0.79/0.58</td>
<td>0.581</td>
<td>0.660</td>
</tr>
</tbody>
</table>

Note: K-M = Kaplan–Meier; LS = “likely stressed” older Dams; US = “likely unstressed” young Dams
There is also a rapidly growing literature that suggests that early life events, especially infectious disease, can contribute to chronic disease late in life (52,53). Another important early life event is the environment experienced within the womb of the female (53). That uterine environment exhibits age-dependent decrements arising from wear and tear as well as the stochastic consequences of reproductive and organismal senescence (54,55). These stochastic changes in a Dam’s physiology alter not only the environment for her eggs but also the eggs themselves. As such, they could have late life consequences for their offspring (eg, [56–59]).

Reproductive senescence has been documented for a variety of insects (60,61) and mammals, including rodents (62). Our results for female offspring are consistent with previous studies on the influence of parental age on offspring fitness. For example, Wang and vom Saal (31) found that offspring of middle-aged first-time CF-1 mothers had lower body weight, delayed maturity, and smaller testes (in males) that could be correlated with differential hormone levels. Tarín and colleagues (36) found that delayed motherhood in hybrids of C57BL/6J and CBA/J strains negatively influences offspring adult body weight and ultimately offspring life expectancy. Thus, there is ample evidence that maternal age has a range of detrimental impacts on offspring fitness. However, as in our study, it is less clear how universal these impacts are and whether they affect male and female offspring in the same way.

There is also a growing body of literature on offspring effects in humans (eg, (63,64)). A long litany of adverse outcomes for both mother and child has been documented for women giving birth under the age of 15 years (65) or beyond the age of 35 years (66,67). For example, advanced maternal age at childbirth is associated with reduced sperm quality (68) and increased infertility rates in sons (69) and menstrual disorders in daughters (70). There are also indications that the age of the father, whether young (71) or old (72), can have adverse outcomes for their child, but the weight of evidence suggests that age of the mother is far more important than that of the father (73). This conclusion is not surprising if the ecological mantra that organisms are a product of their environment is correct; the dramatic age-dependent changes that occur to the ovarian and uterine environments cannot help but have profound impacts on every aspect of the offspring conceived and developed within them.
Our data did not contain the information (eg, physiological markers in either the Dam or her offspring, gender differences in access to suckling among pups, whether maternal behaviors improve with experience) needed to extend and enhance our ability to interpret the results of this study. If maternal experience acquired with successive litters or congenital anomalies in females with truncated reproductive histories influences offspring as could be expected, then maternal effects could emerge at both ends of the reproductive age continuum (for different reasons) and produce a U-shaped risk curve for offspring quality as observed in humans. However, teratogenic effects should not affect our results because our inclusion rules for Dams eliminated those (Dams with fewer than three parities) who potentially experienced these effects.

This study reinforces themes that emerge from the scientific literature, past and present, about the nature of aging. The most fundamental of these themes is the growing recognition that the biological consequences of reproductive and organismal senescence have a sizeable if not dominant stochastic component. The second theme is that gender differences are nearly ubiquitous when studying biological processes. Differences in access to suckling among pups, whether maternal effects could emerge at both ends of the reproductive age continuum (for different reasons) and produce a U-shaped risk curve for offspring quality as observed in humans. However, teratogenic effects should not affect our results because our inclusion rules for Dams eliminated those (Dams with fewer than three parities) who potentially experienced these effects.

Acknowledgments

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