doi:10.1111/evo.12131

PARENTAL AGE, GAMETIC AGE, AND INBREEDING INTERACT TO MODULATE OFFSPRING VIABILITY IN *DROSOPHILA MELANOGASTER*

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Received November 11, 2012
Accepted April 8, 2013

In principle, parental relatedness, parental age, and the age of parental gametes can all influence offspring fitness through inbreeding depression and the parental effects of organismal and postmeiotic gametic senescence. However, little is known about the extent to which these factors interact and contribute to fitness variation. Here, we show that, in *Drosophila melanogaster*, offspring viability is strongly affected by a three-way interaction between parental relatedness, parental age, and gametic age at successive developmental stages. Overall egg-to-adult viability was lowest for offspring produced with old gametes of related, young parents. This overall effect was largely determined at the pupa–adult stage, although three-way interactions between parental relatedness, parental age and gametic age also explained variation in egg hatchability and larva-pupa survival. Controlling for the influence of parental and gametic age, we show that inbreeding depression is negligible for egg hatchability but significant at the larva–pupa and pupa–adult stages. At the pupa–adult stage, where offspring could be sexed, parental relatedness, parental age, and gametic age interacted differently in male and female offspring, with daughters suffering higher inbreeding depression than sons. Collectively, our results demonstrate that the architecture of offspring fitness is strongly influenced by a complex interaction between parental effects, inbreeding depression and offspring sex.

KEY WORDS: **Inbreeding depression, ontogenesis, parental effects, senescence, unguarded X-hypothesis.**

Inbreeding, the mating of related individuals, can depress offspring fitness through the expression of deleterious recessive alleles and loss of heterozygous advantage (Charlesworth and Charlesworth 1987). Recent work has identified inbreeding depression as a fundamental determinant of the dynamics (Keller and Waller 2002; Grady et al. 2006; Walling et al. 2011) and evolution of natural populations (Andersson 2012; Dierkes et al. 2012). The magnitude of inbreeding depression varies with environmental conditions (Armbruster and Reed 2005; Fox and Reed 2011), genetic architecture (Fox et al. 2006), and life-history stages (Charlesworth and Hughes 1996). For example, Charlesworth and Hughes (1996) demonstrated that inbred male fruit flies, *Drosophila melanogaster*, suffer increasing fitness costs as they age, indicating that effects of inbreeding depression can be age dependent. This could be due to the accumulation of late-acting deleterious mutations that are age specific (Charlesworth and Hughes 1996; Reynolds et al. 2007). It is similarly likely that mechanisms of parental senescence might also act as important modulators of the magnitude of inbreeding depression in the offspring, however this hypothesis has received surprisingly little consideration.

Senescence can occur at two distinct levels. At the organismal level, senescence refers to a decline in survival and reproductive ability with advancing age (Rose 1991; Finch and Kirkwood 2000) due to mutation accumulation or antagonistic pleiotropy

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(Medawar 1952; Williams 1957). The mutation accumulation theory proposes that late-acting deleterious mutations tend to accumulate, because of their minimal effects on fitness early in life (Medawar 1952), whereas the antagonistic pleiotropy theory postulates that genes with beneficial effects early in life have pleiotropic deleterious effects on late performance (Williams 1957). At the gametic level, senescence refers to reductions in the fertilizing efficiency and zygote fitness over the postmeiotic lifespan of individual gametes, due to DNA damage of their haploid genome, independently of the ageing of the parental diploid parent. Both organismal and gametic senescence can have profound consequences for offspring fitness (Siva-Jothy 2000; Tarin et al. 2000). It is therefore plausible that the offspring of aged parents and/or aged gametes might be more sensitive to the expression of recessive alleles and loss of heterozygosity imposed by inbreeding.

Here, we experimentally test this hypothesis by measuring the extent to which full-sib mating, parental age, and gametic age interact to determine variation in offspring viability in a laboratory-adapted outbred population of *D. melanogaster*. We measure offspring viability as overall egg-to-adult survival and at individual developmental stages: (i) egg hatchability; (ii) larva-to-pupa survival; and (iii) pupa-to-adult survival. Because inbreeding depression may be sex specific (Saccheri et al. 2005; Bilde et al. 2009), we measured survival separately for sons and daughters at the pupa–adult stage, when phenotypic sexing was possible.

Methods

EXPERIMENTAL POPULATION AND CULTURING

We used a lab-adapted Dahomey wild-type stock of *D. melanogaster*. Flies were maintained at 25°C, in a non-humidified room, on a 12 h:12 h light:dark cycle, and fed standard sugar–yeast–maize–molasses medium with excess live yeast granules (Lewis 1960). The stock has been maintained since 1970 in four large (several thousand flies), outbred population cages (Partridge and Farquhar 1983) of dimensions 30 cm × 15 cm × 20 cm. Each population was fed with three bottles of food medium per week. These four populations were mixed into one single large population approximately 1 year prior to experiments to promote genetic variability in our experimental flies. This stock exhibits substantial levels of genetic variation (Wilkinson et al. 1990; Whitlock and Fowler 1996; Fowler et al. 1997), contains selectable variation for a range of life-history, behavioral, and physiological traits (Wigby and Chapman 2004; Wigby et al. 2009), and displays some levels of inbreeding depression after full-sib mating (Fowler and Whitlock 1999; Mooers et al. 1999; Tan et al. 2012). The Dahomey stock is maintained with overlapping generations to minimize artificial selection on replication rate and life span.

To obtain parents of the experimental flies, eggs were collected and raised at standard density (∼100 flies per bottle; Clancy and Kennington 2001). Parental flies were collected as virgins within 8 h of eclosion using ice anesthesia, aged for 1 week before single males and females were paired in vials to produce families. The parental pair was discarded after 24 h and the eggs left to develop. Virgin adults emerging from these vials were used for experimental trials.

EXPERIMENTAL PROCEDURE

One-day post-eclosion (young) females were paired with, and mated to, single males of the same age. Males were either full-siblings (related [R]; $n = 40$) or nonsiblings (unrelated [U]; $n = 40$) of the female. The pairs were independent of one another, for example, the male and female from the related pair are from family 1 and the male and female from the unrelated pair belong to different families and are not obtained from family 1. Males were then discarded and females allowed to oviposit for 24 h in individual vials (1-day post-copulation; young). To investigate the effect of gametic senescence on offspring viability, females were transferred to vials where they were deprived of an egg-laying substrate (sugar–yeast—maize–molasses medium) for 15 days. We ensured the survival of the females by providing a smear of Baker's yeast dried onto the side the vial, and 3 mL of saturated sucrose solution placed onto cotton wool inserted at the bottom of the vial (Partridge et al. 1987). The cotton wool was kept moist all the time by resupplying saturated sucrose solution on alternate days. These conditions discourage females from ovipositing (Partridge et al. 1987), thereby reducing sperm utilization rates (Trevitt et al. 1988). Therefore, sperm and eggs were retained, and aged, in the female reproductive organs. After 15 days, females were transferred to fresh vials with egg-laying substrate and allowed to oviposit for 24 h before they were discarded (15 days post-copulation; old). To examine the potential effects of parental age on offspring mortality, we repeated the above protocol with 15 days post-eclosion (old) virgin females and males from the same set of families ($n = 40$ related pairs; $n = 40$ unrelated pairs). Namely, females were kept in vials with egg-laying substrate prior to mating on day 15 post-eclosion. Males were removed post-copulation and females allowed to oviposit in individual vials for 24 h. Therefore, in this treatment, parents were 15 days post-eclosion and gametes were 1 day post-copulation. Females were then transferred to vials without oviposition substrate for 15 days. Thirty days after eclosion and 15 days post-copulation, females were placed in vials with egg-laying substrate for 24 h (Table 1). Because the first mating of virgin females causes the initial release of mature eggs (Chapman et al. 2001), eggs laid by females on day 15 post-copulation would be, at most, 15-days old. Therefore, a comparison of offspring produced by this treatment with that produced by 1-day post-eclosion parents, 15-days

Table 1. Parental organismal age across experimental treatments.

Treatments	Age at copulation	Age at egg-laying
Young parents, young gametes	1-day-old	1-day-old
Young parents, old gametes	1-day-old	15-days-old
Old parents, young gametes	15-days-old	15-days-old
Old parents, old gametes	15-days-old	30-days-old

post-copulation gametes allows us to examine the effect of parental age while controlling for gametic age. Altogether, we conducted 320 trials (i.e., 80 pairs × two gametic age treatments × two parental age treatments). Throughout the article, we use the terms "young parents" for individuals that copulated 1-day post-eclosion; "old parents" for individuals that copulated on day 15 post-eclosion; "related" for full-sib matings; "unrelated" for non-sib matings; "young gametes" for gametes contributing to zygotes produced 1-day post-copulation; and "old gametes" for gametes contributing to zygotes produced 15 days post-copulation (Table 1).

To quantify offspring viability at different development stages, we monitored the number of eggs, larvae, pupae, and adults produced by individual females. We counted the number of eggs after the females were removed from the vials. Twenty-four hours later, after the viable eggs had hatched, we counted the number of unhatched eggs. We also quantified the number of noneclosed pupae and adults 12 days after the oviposition period. Because the majority of the flies eclosed 10 days after oviposition, allowing 12 days before counting provided ample time for development. To quantify sex-specific differences in mortality, we also determined the sex of individuals from the pupae and adult stage (but not at the egg and larval stages). Pupae were sexed based on the presence/absence of male-specific foreleg sex combs after opening pupal cases with fine forceps.

For ease of interpreting the results, we also calculated the coefficient of inbreeding depression δ (Lande and Schemske 1985) at each developmental stage:

$$\delta = 1 - (X_I/X_O),$$

where X_I is the inbred viability and X_O is the outbred viability. Therefore, a higher inbreeding depression coefficient indicates a higher magnitude of inbreeding depression.

STATISTICAL ANALYSIS

To analyze the effects of parental relatedness, parental organismal age, and gametic age on offspring viability, we first considered variation in egg–adult viability, and then considered variation in viability at specific developmental stages: egg hatchability (proportion of eggs that hatched), larva–pupa viability (proportion of larvae that developed to pupae), and pupa–adult viability (proportion of pupae that developed to adults). The proportion of individuals that developed into the next stage in each vial was thus a unit of replication. Egg–adult viability is widely used as an indication of overall viability in inbreeding studies (Lopez-Fanjul and Villaverde 1989; Mack et al. 2002), whereas the other response variables were used to better understand the ontogenetic mechanisms through which parental relatedness, parental age, and gametic age might affect offspring viability.

There was no effect of parental relatedness, parental age, and gametic age on the number of eggs laid by females (Table S1). Also, we found no overall difference in variance between the inbred and outbred treatment (F-test, $F_{39,39} = 1.07$, $P = 0.416$), suggesting that variability in environmental conditions between treatments was small. Therefore, we did not adjust viability measures for egg numbers or variance. We analyzed variation in (i) overall egg–adult viability, (ii) egg hatchability, and (iii) larva–pupa viability with three separate generalized linear mixed models (GLMMs) with binomial error distribution, parental age, parental relatedness, and gametic age, and their two- and three-way interactions as fixed factors, and family identity as a random variable (80 families in all; Online Material SI). Finally, we analyzed variation in (iv) pupa–adult viability through a GLMM with binomial error distribution, pupa–adult viability as the response variable, parental relatedness, parental organismal age, gametic age, offspring sex, and their interactions as fixed factors, and family identity as a random variable. The significance of the fixed factors was assessed using the likelihood-ratio test on models with and without the fixed factor (Valdar et al. 2006; Ockinger et al. 2010). For each test (i)–(iv) we used an information theoretic approach with corrected Akaike's information criterion (AIC) to select models. Models in which $\Delta AIC \leq 2$ were retained (i.e., there is no strong indication that one model has the best fit; Table S2). R version 2.15.0 was used for analysis of data (Online Material SII).

Results

EGG–ADULT VIABILITY

We identified one model that was effective (lowest ΔAIC) at explaining variation in egg-to-adult viability, with a significant three-way interaction between parental relatedness, parental organismal age, and parental gametic age (Table 2A; Table S2). This model indicates that the viability of outbred offspring produced with young gametes was higher than the viability of outbred offspring produced with old gametes, particularly so in old rather than young parents (Fig. 1A), resulting in the offspring of young parents and old gametes suffering the highest inbreeding depression coefficient (Fig. 1B).

Table 2. Effects of parental relatedness, parental age, and gametic age on (A) egg–adult viability; (B) egg hatchability; (C) larva–pupa viability; (D) sex-specific pupa–adult viability. Gametic age: young = 1-day post-copulation and old = 15 days post-copulation. Parental age at copulation: young = 1-day post-eclosion and old = 15 days post-eclosion.

Response variable	Factors	Mean ± SE	df	χ^2	*P*
A. Egg–adult viability	Parental relatedness	Related: 0.592 ± 0.020; unrelated: 0.722 ± 0.020	1	16.29	<0.001
	Parental age	Young: 0.666 ± 0.019; old: 0.648 ± 0.022	1	9.44	0.002
	Gametic age	Young: 0.724 ± 0.018; old: 0.588 ± 0.021	1	261.28	<0.001
	Parental relatedness × Parental age		1	37.37	<0.001
	Parental relatedness × Gametic age		1	6.67	0.010
	Parental age × Gametic age		1	8.67	0.003
	Parental age × Gametic age × Parental relatedness		1	8.38	0.003
B. Egg hatchability	Parental relatedness	Related: 0.815 ± 0.019; unrelated: 0.840 ± 0.019	1	0.87	0.351
	Parental age	Young: 0.865 ± 0.016; old: 0.791 ± 0.021	1	161.11	<0.001
	Gametic age	Young: 0.905 ± 0.011; old: 0.748 ± 0.023	1	477.74	<0.001
	Parental relatedness × Parental age		1	39.82	<0.001
	Parental relatedness × Gametic age		1	3.88	0.049
	Parental age × Gametic age		1	6.25	0.012
	Parental age × Gametic age × Parental relatedness		1	4.00	0.046
C. Larva–pupa viability	Parental relatedness	Related: 0.832 ± 0.017; unrelated: 0.916 ± 0.010	1	12.36	<0.001
	Parental age	Young: 0.859 ± 0.016; old: 0.888 ± 0.013	1	4.03	0.045
	Gametic age	Young: 0.862 ± 0.016; old: 0.885 ± 0.012	1	12.00	0.001
	Parental relatedness × Parental age		1	4.75	0.029
	Parental relatedness × Gametic age		1	8.65	0.003
	Parental age × Gametic age		1	10.59	0.001
	Parental age × Gametic age × Parental relatedness		1	4.90	0.027
D. Pupa–adult viability	Parental relatedness	Related: 0.898 ± 0.009; unrelated: 0.946 ± 0.006	1	13.19	<0.001
	Parental age	Young: 0.881 ± 008; old: 0.920 ± 0.008	1	26.28	<0.001
	Gametic age	Young: 0.923 ± 0.007; old: 0.877 ± 0.009	1	63.55	<0.001
	Sex	Male: 0.905 ± 0.009; female: 0.897 ± 0.008	1	5.21	0.022
	Parental relatedness × Parental age		1	12.32	<0.001
	Parental relatedness × Gametic age		1	10.79	0.001
	Parental relatedness × Sex		1	4.60	0.032
	Parental age × Gametic age		1	19.38	<0.001
	Parental age × Sex		1	0.18	0.676
	Gametic age × Sex		1	0.44	0.506
	Parental relatedness × Parental age × Gametic age		1	6.73	0.009
	Parental relatedness × Parental age × Sex		1	0.86	0.354
	Parental relatedness × Sex × Gametic age		1	0.06	0.813
	Parental age × Gametic age × Sex		1	5.65	0.017
	Parental relatedness × Parental age × Gametic age × Sex		1	3.90	0.048

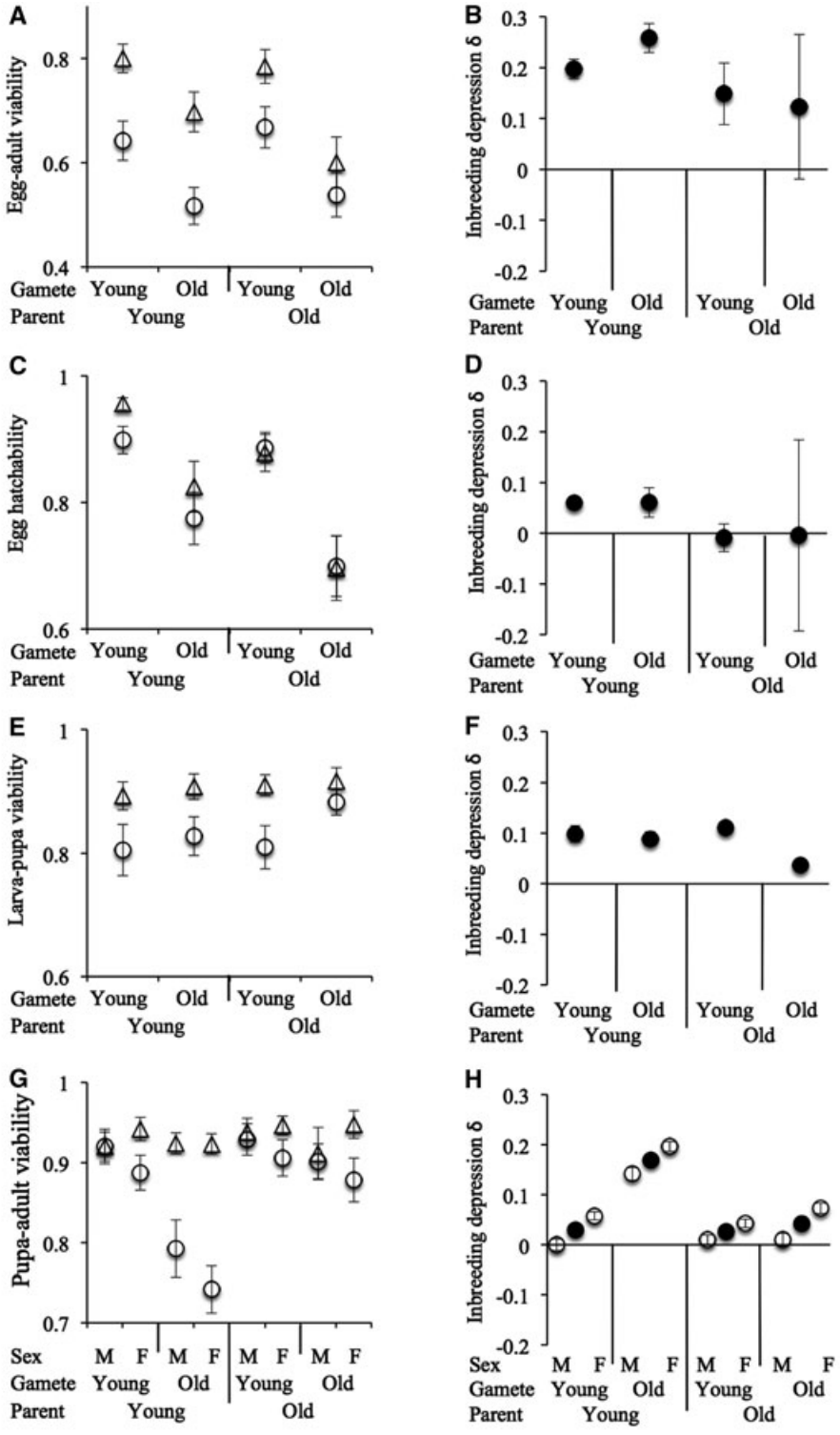

Figure 1. Viability of offspring produced by young and old, related and unrelated parents at different developmental stages (left column) and coefficient of inbreeding depression (δ; right column). (A, B) Cumulative egg–adult viability; (C, D) egg hatchability; (E, F) Larva–pupa viability; (G, H) pupa–adult viability. For parts A, C, E, and G, triangular symbols represent viability when parents are unrelated; circular symbols represent viability when parents are related. Unfilled symbols for part H denote δ for the separate sexes whereas filled symbol denote mean δ for both sexes. "Parent" refers to parental age and "Gamete" refers to gametic age. Results are presented in Table 2. The error bars for the graphs in the left column denote the standard errors of the mean viability whereas those in the right column denote the standard errors of the mean inbreeding coefficient of 500 values obtained via bootstrapping.

EGG HATCHABILITY

The patterns of variation in egg–adult viability (above) were largely mirrored by patterns of variation in egg hatchability. We identified a significant three-way interaction between parental relatedness, parental organismal age, and gametic age (Tables 2B and S2), whereby hatchability was higher in zygotes produced with young gametes, particularly so in young unrelated parents (Fig. 1C). As a result, evidence of inbreeding depression was limited to the eggs of young parents (Fig. 1D).

LARVA–PUPA VIABILITY

Again, the best model detected a significant three-way interaction on variation in larva–pupa viability between parental relatedness, parental organismal age, and gametic age (Tables 2C and S2). Inbred offspring generally suffered lower viability than outbred offspring (Fig. 1E). However, in this case inbreeding depression was largest in the offspring of old parents and young gametes, and smallest in the offspring of old parents and old gametes (Fig. 1F).

PUPA–ADULT VIABILITY AND SEX-SPECIFIC EFFECTS

The best model detected a significant four-way interaction between parental relatedness, parental organismal age, parental gametic age, and offspring sex (Tables 2D and S2). The magnitude of inbreeding depression was substantially higher for old gametes than for young gametes among young parents, and slightly lower for old gametes than for young gametes among old parents group (Fig. 1G, H). Consistent with the idea that the magnitude of inbreeding depression differs with offspring sex, the coefficient of inbreeding depression of daughters was more than twice that of sons (Parental relatedness $\times$ Sex, $P = 0.019$; Daughters, $\delta = 0.091$; Sons, $\delta = 0.041$; Fig. 1H). This resulted in a significant four-way interaction between offspring sex, parental relatedness, parental organismal age, and gametic age (Table 2D; Fig. 1G, H).

Discussion

Previous studies of *D. melanogaster* have demonstrated the independent effects of inbreeding depression, for example in terms of decreased offspring fertility and viability (Partridge et al. 1985; Vermeulen and Bijlsma 2004), and parental ageing, for example, in terms of reduced offspring fertility (David et al. 1975; Economos et al. 1979). The aim of our study was to test the extent to which inbreeding depression is modulated by organismal and gametic mechanisms of parental ageing. We found that variation in offspring mortality at all developmental stages and cumulative egg–adult viability is strongly influenced by a three-way interaction between parental relatedness, parental organismal age, and gametic age. In terms of overall egg–adult viability, the magnitude of inbreeding depression suffered by the offspring of senesced gametes and young parents is approximately two times that of the offspring of young gametes and young parents, and those of gametes of both ages of old parents. Thus, gametic ageing appears to exacerbate the effects of inbreeding in young parents but not in old parents. One potential reason for this dichotomy might be that older parents increase investment in offspring, for example by increasing egg resources, to offset the effects of inbreeding. However, this hypothesis remains to be tested. It is also important to note that the relationship between viability and the interaction among parental age, parental relatedness, and gametic age, varies with developmental stage. The deleterious mutations that cause inbreeding depression and senescence may manifest at different development stages (Schupbach and Wieschaus 1986; Hurd and Saxton 1996), potentially modifying the relative effects of parental age and gametic age on offspring viability.

The influence of parental gametic age was strong across all developmental stages. Our experimental design measured the effect of gametic age by comparing the viability of inbred and outbred offspring produced on day 1 and 15 post-copulation. The egg deposition rates of females were suppressed by deprivation of suitable oviposition sites, subsequently reducing sperm depletion rate Trevitt et al. 1988). Therefore, this design captures the effect of sperm senescence occurring during female sperm storage. The effect of post-meiotic sperm senescence is beginning to emerge as a significant determinant in offspring viability in natural populations (White et al. 2008). Because females were prevented from remating and from ovipositing between day 1 and 15 post-insemination, it is likely that a proportion of these eggs may have been ovulated days in advance of oviposition. The retention of mature eggs in the ovary, which can be induced by dietary restriction (Drummond-Barbosa and Spradling 2001), inhibits the development of younger oocytes in *D. melanogaster* (Meola and Lea 1972). It has been suggested that these oocytes would therefore undergo cell death to recycle macromolecules and prevent blockage of the ovarioles (Chao and Nagoshi 1999; Buszczak and Cooley 2000). Therefore, we cannot rule out the possibility that eggs laid on day 15 will be a mixture of newly produced eggs and old eggs, suggesting that egg senescence may also contribute to explain the effect of gametic age. Further studies should aim to disentangle the effects of egg and sperm age on inbreeding depression.

To our knowledge, only two other studies, of humans and seed beetles, *Callosobruchus maculatus*, have examined how inbreeding depression in offspring fitness traits change with maternal age (Yaqoob et al. 1998; Fox and Reed 2010). In these studies, the magnitude of inbreeding depression increased with maternal age, contrary to our study. In our study, the reduced inbreeding depression in older parents is largely caused by a decreased difference in egg hatchability between related and unrelated parents. One possibility is that this result may have been

due to the selective disappearance of families with reduced egg hatchability in older parents. However, we found no evidence of this as none of the parental flies died during the experiment and thus all family lines were represented across all age treatments (data not shown). A more plausible explanation might be that the large effects of parental senescence on fertility and/or prehatching mortality might dominate that of inbreeding, and potentially mask differences between inbred and outbred offspring. Declines in sperm production and genetic quality in old males (Pizzari et al. 2008; Dean et al. 2010), as well as an increase in the levels of oxidatively damaged proteins in the eggs (Fredriksson et al. 2012), can result in lower fertilizing efficiency and/or embryonic viability. Such mechanistic effects may occur independently of the expression of recessive mutations during inbreeding and mask the effects of increased homozygosity, potentially explaining the decreased magnitude of inbreeding depression in old parents. These results highlight the potential importance of interactions between inbreeding and parental age effects.

VARIABILITY OF INBREEDING EFFECTS AT DIFFERENT DEVELOPMENTAL STAGES

Although there was an overall decrease in egg–adult viability due to inbreeding, this was mainly mediated by larva–pupa and pupa–adult viability, not by egg hatchability. This is consistent with a previous study of *D. melanogaster*, which found that egg hatchability was only slightly affected by inbreeding ($< 2\%$), but relative larval–adult survival was reduced by 20% (Enders and Nunney 2010). The delayed detrimental effect of inbreeding suggests that the number of genes involved in early development might be low, therefore reducing the probability that mutations are expressed. In addition, we found an overall decline in mortality, in both inbred and outbred offspring, from the egg to adult stage, consistent with the evolutionary demography of ontogenesis in which the death rate of each cohort tends to decrease with increasing age between conception and maturity (Levitis 2011). High mortality during egg hatchability may have masked the ability to detect the effects of inbreeding.

INBREEDING DEPRESSION AND SEX-SPECIFIC EFFECTS

Our results indicate that the effects of inbreeding vary with offspring sex and developmental stages. The "unguarded-X" hypothesis posits that the heterogametic sex should suffer less from inbreeding compared to the homogametic sex (i.e., females here), because inbreeding will increase the risk of expression of any X-linked recessive deleterious allele only in the heterogametic sex. We found that inbreeding depression, was higher for the homogametic sex, which is in accordance with this theory and consistent with previous studies on inbreeding depression in the longevity of insects (Saccheri et al. 2005; Bilde et al. 2009), birds (Keller et al. 2008), and mammals (Coulson et al. 1999; Rioux-Paquette et al. 2011). Interestingly however, the reversed effect, where the heterogametic sex suffers more from inbreeding depression, is often observed in reproductive traits such as reproductive success in *D. melanogaster* (Millers and Hedrick 1993; Enders and Nunney 2010), fertility in the butterfly *Bicyclus anynana* (Saccheri et al. 2005), fledging success in Takahes, *Porphyrio hochstetteri* (Jamieson et al. 2003), and hatching rate in song sparrows, *Melospiza melodia* (Keller 1998). Why the difference in sex-differential effects of inbreeding depression in survival and reproduction is unknown. Because we could not determine the sex of dead embryos or larvae, nor measure the adult reproductive success of the inbred *versus* outbred offspring, we cannot determine sex-specific mortality prior to the pupae stage, or during adulthood, meaning that we can make only limited conclusions about sex-specific inbreeding load at different life-history stages.

Conclusions

Our study indicates that inbreeding depression is sex-specific and modulated by parental effects such as parental organismal age and parental postmeiotic gametic age. The variation in offspring viability at different development stages with these factors demonstrates the importance of considering the various life-history stages when studying the fitness costs of inbreeding. Our results suggest inbreeding may often harm the offspring of young individuals to a higher magnitude, particularly in females of senesced gametes. Ultimately, further elucidating the genetic underpinnings of such variations in fertility and mortality will provide important insights into the relationship between inbreeding, senescence, and sex.

ACKNOWLEDGMENTS

The authors thank Rebecca Dean for comments on the manuscript. This work was funded by an Elizabeth Hannah Jenkinson fund awarded to CT and a Philip Leverhulme Prize to TP. The authors declare no conflict of interest.

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Associate Editor: M. Johnston

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Supplementary Online Material SI. R code used in analysis.

Supplementary Online Material SII.

Table S1. Effects of parental relatedness, parental age, and gametic age on egg numbers.

Table S2. Akaike's information criterion (AIC) of generalized linear mixed models for the different response variables.