**ARTICLE**

**Offspring provisioning explains clone specific maternal age effects on life history and lifespan in the water flea, *Daphnia pulex***

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**ABSTRACT**

Genetic inheritance underpins evolutionary theories of aging, but the role that non-genetic inheritance plays is unclear. Parental age reduces the lifespan of offspring in a diverse array of taxa but has not been explained from an evolutionary perspective. We quantified the effect that maternal age had on the growth and maturation decisions, life history, rates of senescence and lifespan of offspring from three *Daphnia pulex* clones collected from different populations. We then used that data to test general hypotheses proposed to explain maternal age effects on offspring lifespan. Three generations of breeding from young or old mothers produced dramatic differences in the life-histories of fourth generation offspring, including significant reductions in lifespan. The magnitude of the effect differed between clones suggesting that genetic and non-genetic factors ultimately underpin trait inheritance and shape patterns of aging. Older parents did not transmit a senescent state to their offspring. Instead, offspring from older ancestors had increased early-life reproductive effort, which resulted in an earlier onset of reproductive senescence, and an increased rate of actuarial senescence that shortened their lifespan. Our results provide a clear example of the need to consider multiple inheritance mechanisms when studying trait evolution.

**INTRODUCTION**

Aging or senescence is the intrinsic deterioration of an organism with age, leading to decreased physiological functioning and ultimately death (Flatt and Schmidt 2009; Medawar 1952; Partridge 2009; Partridge and Gems 2006; Rose 1991; Williams 1957). Evolutionary theories of aging predict that between-individual differences in rates of senescence should have a heritable genetic basis (reviewed in Rose 1991); a hypothesis that is supported by the results of numerous laboratory-based experiments (reviewed in Flatt and Schmidt 2009; Partridge 2009) and evidence from wild populations (Charmantier et al. 2006; Wilson et al. 2007). However, there is an increasing realisation that parents may also alter the phenotypes of their offspring via non-genetic inheritance mechanisms such as the transmission of epigenetic variation, the transmission of plastic phenotypes (acquired traits), and the effects of parental environment and state on offspring phenotype (Bonduriansky 2012; Bonduriansky and Day 2009; Danchin 2013; Danchin et al. 2011; Danchin and Wagner 2010; Hallsson et al. 2012; Uller 2008). But we are only just beginning to investigate how genetic and non-genetic inheritance mechanisms combine to shape offspring traits (Badyaev and Uller 2009; Bonduriansky and Day 2009; Danchin 2013; Hallsson et al. 2012; Townley and Ezard 2013; Uller 2013).

Parental age effects on offspring life-histories offer an example of non-genetic inheritance that is especially interesting with respect to the evolution of aging and lifespan (Priest et al. 2002). Evolutionary theories do not typically assume that the age of the parent has any proximate influence on rates of senescence of offspring (but see Kong et al. 2012), yet offspring from older parents have been found to have a shorter lifespan in taxa as diverse as rotifers, duckweed, houseflies, stink bugs, fruit flies, flour beetles, mealworms, nematodes, yeast and humans for over a hundred years (reviewed in Fox et al. 2003; Priest et al. 2002). This pattern is termed "The Lansing Effect" after Albert Lansing (1947; 1948; 1954) who demonstrated that rearing successive generations of clonal rotifers, *Philodina citrina* and *Euclanis triquetra* from old mothers dramatically reduced offspring longevity. There is still no accepted explanation for this effect (Priest et al. 2002). Moreover, the validity and general importance of Lansing's findings have repeatedly been questioned (Comfort 1953; King 1983; Rose 1991) despite the fact that supportive evidence continues to accumulate (Beardmore and Shami 1985; Fox et al. 2003; Gillespie et al. 2013; Hercus and Hoffmann 2000a; Jennings and Lynch 1928; King 1983; Lansing 1947; Lansing 1948; Lansing 1954; Lints and Hoste 1974; Lints and Hoste 1977; Murphy and Davidoff 1972; Priest et al. 2002; Tarín et al. 2005). Reservations about the Lansing effect derive from Lansing’s (1947; 1948; 1954) failure to identify the mechanism underpinning the effect, and subsequent studies focussing on testing for a Lansing effect, rather than explaining it (but see King 1983 for an exception). Previous studies have linked parental age with altered reproductive schedules (Bouwhuis et al. 2010; King 1983), altered mortality rates (Priest et al. 2002), altered investment in offspring (reviewed in Marshall et al. 2010), and offspring viability (Benton et al. 2008; Hercus and Hoffmann 2000b; Kern et al. 2001; Priest et al. 2002; Reid et al. 2010; Tarín et al. 2005). But no study has ever simultaneously linked parental age, patterns of offspring development, offspring life histories and rates of aging. Here we collect this data in order to test two general hypotheses that could explain how parental age effects (including the Lansing effect) have evolved as a form of non-genetic inheritance.

The senescent parent hypothesis, proposes that offspring from older parents die sooner because they transmit their physiological deterioration to their offspring, either via a direct reduction in the provisioning of offspring, a deterioration of the offspring's developmental environment, or the transmission of a higher mutation load (Kong et al. 2012). The hypothesis is supported by studies that have demonstrated that older, senescent parents produce offspring with reduced fitness attributes (Diaz and Esponda 2004; Gillespie et al. 2013; Kern et al. 2001; Tarín et al. 2005) and predicts that offspring from older mothers are born with an elevated ‘biological age’ meaning that they have higher age-independent mortality rates (frailty), lower age-independent reproductive potential and lower growth rates.

In addition, the non-mutually exclusive offspring response hypothesis proposes that offspring from older parents die sooner because the plastic responses they adopt to counteract parental age-related changes in offspring provisioning are costly. If older parents provision offspring less, offspring might employ compensatory strategies such as catch-up growth (Boersma and Wit 1997) that lead to an earlier onset, or an accelerated rate of senescence and a shortened lifespan (Metcalfe and Monaghan 2001; Monaghan 2008). Alternatively, they might adopt a terminal investment like strategy (Cluttonbrock 1984; Williams 1966) that increases early-life reproductive effort but also induces costs that shorten the lifespan (Descamps et al. 2008; Gustafsson and Pärt 1990; Massot et al. 2011; Nussey et al. 2006; Reed et al. 2008; Reid et al. 2003). A reduction in offspring lifespans could also derive from mothers that invest more in their offspring as they age (see Marshall et al. 2010 for a review of causes and examples of positive relationships between maternal age and offspring size) if a head start in life facilitates faster growth rates and increased early-life reproductive effort (Ebert 1994; Fox and Czesak 2000).

Irrespective of whether parental reproductive investment increases or decreases with age, the offspring response hypothesis predicts that the offspring from older mother will have increased growth rates and/or increased early life reproductive effort, and an earlier onset, or an accelerated rate of actuarial and/or reproductive senescence that shortens offspring lifespan. The hypothesis is supported by studies linking increased investment in early growth and reproduction with late-life costs (Metcalfe and Monaghan 2001; Monaghan 2008) and proximate mechanisms linked with increased rates of senescence such as oxidative stress (Monaghan et al. 2009), telomere loss (Hall et al. 2004; Houben et al. 2008; Monaghan and Haussmann 2006) and stress responses (McEwen 2007). Exactly how parental age translates into offspring with increased early life reproductive effort is unclear. But the size and age at which individuals mature may play a key role as body size often constrains fecundity (Roff 1992). Our recent finding that the 'decision' to mature (modelled as a probabilistic maturation reaction norm, PMRN) is itself plastic (Harney et al. 2012) raises the possibility that parental age may alter the maturation decisions of their offspring as well as influencing growth rates, although this hypothesis has never been tested.

The four objectives of this study were therefore to (1) quantify the effect that non-genetic inheritance, here manifested through maternal age effects, has on the life-history, lifespan and rates of actuarial and reproductive senescence of *Daphnia pulex*, (2) distinguish between the two hypotheses we have proposed to explain the Lansing effect (3) determine whether the pattern and the magnitude of non-genetic inheritance effects varies between clones and (4) determine whether a plastic response of offspring to maternal age related changes in offspring provisioning - predicted by the offspring response hypothesis - includes a plastic adjustment in the age and/or size at which offspring decide to mature. The first three objectives were tested in an experiment using three *Daphnia pulex* clones isolated from three different populations. We repeatedly bred from young or old mothers for three generations in order to create maternal age lines (see Fig. 1A). We then reared offspring from the fourth generation and quantified the effect that genotype and three generations of maternal age inheritance had on offspring size, growth rate, age and size at maturity, age-specific reproductive effort, age-specific mortality rate (hazard rate) and survival. The fourth objective was tested using a separate experiment (see Fig. 1B) because maturation decisions are modelled as a process or reaction norm rather than as an individual trait (Heino et al. 2002; Van Dooren et al. 2005) and require a range of growth trajectories for their quantification (Harney et al. 2012). The second experiment provides an independent test of the hypothesis that maternal age influences offspring life histories, but over a single generation, rather than over accumulated generations.

**METHODS**

*Experimental animals*

*Daphnia pulex* clones used in this study were isolated from various UK sites. Clone NBG70 came from a pond in Ness Botanical Gardens, Ness, Merseyside, UK (53°16’16’’N, 03°02’47’’W), clone Boris came from a pond in Sheffield, South Yorkshire, UK (53°24’18’’N, 01°27’27’’W) and clone D8.7A came from a pond near Corfe Castle in Dorset, UK (50°38′33”N, 02°05′58”W). Since being isolated, the clones have been maintained in incubators at 21±1°C on a 14:10 light: dark cycle kept in hard artificial pond water media, ASTM (OECD, 1984) enriched with a standard organic extract (Baird et al., 1989).

*Experiment 1 - genotype and maternal age line effects on offspring life-history, lifespan and rates of senescence.*

The experimental design is outlined in Figure 1A. All animals in the experiment were reared in individual 200ml glass jars containing 150ml of ASTM enriched with a standard organic extract (Baird et al., 1989), replaced every other day and fed high food (200,000 cells ml-1 day-1 of batch-cultured *Chlorella vulgaris*,quantified with a haemocytometer). For each clone, a single female was isolated from stocks, and reared on high food until she produced at least three offspring in a clutch. From that clutch, three offspring were randomly selected and reared individually to become the parental generation (see Fig 1A). Young and old maternal lines were set up from the parental generation using offspring from mothers' first clutches as the young maternal line and offspring from their fifth clutches as the old maternal line. In order to minimize the possibility that within clone lineage effects (Sakwinska 2004) or clonal selection of *de novo* mutations or epimutations could explain any of our treatments effects we set up each new generation of the experiment with randomly selected offspring from multiple mothers that produced offspring within a 12 hour period (see Fig. 1 a,b). On occasion, offspring may have come from less than three mothers (not recorded) if mothers did not produce offspring at the same time. However, systematic bias due to female mortality was unlikley because almost all mothers survived to 5th clutch during the set-up of the experiment (see Table S1). By the end of the experiment, fourth generation offspring had mothers, grandmothers and great-grandmothers that had all come from first or fifth clutches (see Fig. 1A). The life history, lifespan and rates of senescence of these fourth generation offspring were then compared.

For each clone, fifteen first clutch offspring from the young maternal line and fifteen fifth clutch offspring from the old maternal line were randomly selected from the pooled offspring of six mothers that had dropped their clutches within a twelve hour window. All individuals were photographed as neonates and then every time they moulted throughout their life, using a Canon EOS 350D digital camera connected to a Leica MZ6 dissecting microscope. Body size was measured as the distance from the top of the head to the base of the tail spine using the image analysis software, ImageJ version 1.45s (Rasband, 1997). Pre-maturation growth rates for each individual were estimated as the best limited unbiased predictors (BLUP's) from a linear mixed effects model with size as the response variable, age as a covariate and individual fitted as a random term for intercept and slope (Crawley 2002). Individuals were recorded as being mature once eggs were observed in the brood pouch. We counted the number of offspring each individual produced in each clutch for the rest of their lives until the day of death.

*Experiment 1 - Statistics*

The effect that clone and maternal age line had on the life-history and lifespan of fourth generation offspring was compared using a multivariate analysis of variance (MANOVA) with neonate size, growth rate (intercept), growth rate (slope), size at maturity, age at maturity, lifetime fecundity, and lifespan as response variables and clone (Boris, D87A, NBG70) and maternal age line (Young, Old) fitted as fixed factors. Differences were then visualized and interpreted using a principal component analysis (PCA) to summarize how maternal age line altered the life histories of 4th generation offspring. Since PCA’s cannot be used for hypothesis testing, pairwise differences in clone responses to maternal age line treatment were tested by comparing the length and angle of phenotypic change vectors following the methodology of Collyer and Adams (2007).

The data collected in experiment 1 were also used to compare the age-specific reproduction and mortality rate of offspring from young and old maternal age lines. Data for all three clones were combined in order to maximise sample size and statistical power, but clone by treatment interactions were also tested in a subsequent analysis. Age-specific reproduction was modelled using generalized additive mixed models (GAMM's) implemented using the gam function from the mgcv library version 1.7-23 (Wood 2011) in R version 3.0.1 but with random effects incorporated as penalized regression terms (Wood 2011). In all models, the default settings of the gam package were used, with the number of knots (*k*) being estimated as part of the fitting procedure (Wood 2011). After fitting a simplistic model with the same smoothing function for age for all individuals, and individual included as a random intercept and smoothing function, the effect of maternal age line, clone, and interactions between them were assessed using AIC and by statistically comparing the residual variance explained by models of increasing complexity using *F* statistics (Crawley 2002; Wood 2011). Following Jones *et al.* (2008), the age at the onset of senescence was estimated for each group as the point of peak fecundity predicted by the GAMM. Differences in the rate of reproductive senescence were then tested using a linear mixed effects model with clutch size following the onset of senescence fitted as a response variable, age after the onset of senescence fitted as a covariate, maternal age line fitted as a fixed factor and individual fitted as a random term.

Age-specific mortality rates were compared by fitting parametric survival models implemented using the flexsurvreg function within the flexsurv package version 0.3 in R 3.1.0. Note that mortality rates cannot be unambiguously measured directly from the data, since each death could be interpreted as an instantaneous burst of infinite mortality rate. All we can do is infer mortality rates by fitting a survival model to the data, or estimate them by binning the survival events (as in Bronikowski and Flatt 2010). Since we have only 10-15 individuals per clone/treatment combination, binning the data does not provide enough mortality rate: age combinations to make any robust inferences. Nevertheless, there are enough points to fit survival models directly to the (un-binned) data, and to test hypotheses about how these depend on inferred mortality rates, clone and treatment.

A suite of survival distributions (exponential, Weibull, Gompertz, and piecewise-linear hazard) were fitted, under the assumption that the parameters of the distribution depended on the treatment (maternal age line) and on clone, and the Akaike Information Criterion corrected for small sample sizes (AICc) used to choose the distribution to use in the analysis. Likelihood ratio tests (LRT) were used to investigate how the survival curves depended on the treatment and/or the clone, assuming that the likelihood ratio followed a chi-squared distribution. Survival was assumed to depend on maternal age line when testing for the effect of clone, and vice versa. A p-value is not given for the interaction between these factors, because there is no meaningful way to have direct effects but no interaction in this type of model. This is because the absence of an “interaction” would mean that the parameters could be expressed as the sum of separate effects from the different explanatory variables, but this has no particular biological significance since the hazard depends on these parameters in a non-linear way.

*Experiment 2 - genotype and maternal age effects on growth and maturation decisions*

We ran a separate experiment alongside experiment 1 to test the hypothesis that maternal age affects the maturation decisions of offspring in *D. pulex* (see Fig. 1B). Three replicate offspring from each clone (Parental generation females in Fig.1B) were individually reared to maturity on high food (200,000 cells ml-1 day-1 *C. vulgaris*) as above. In order to remove any unwanted maternal effects, and to condition animals to their current environment, clones were conditioned for three generations using third clutch offspring to set up each new generation (see Fig 1B). In the F4 generation, the number of mothers used to set up the experiment was increased to eight to ensure enough offspring would be available (see Table S1 for the data on the mortality of mothers used to set up the experiment). Thirty randomly selected offspring from young mothers (clutch 1) and thirty randomly selected offspring from old mothers (clutch 11) were isolated for each clone and fifteen were reared individually on high food (200,000 cells ml-1 day-1 *C. vulgaris*) while the other fifteen were reared individually on low food (40,000 cells ml-1 day-1 *C. vulgaris*). Offspring were photographed as neonates and after every moult and measured as in experiment 1. Size and age data were collected until offspring had dropped their first clutch.

*Experiment 2- statistics*

The pre-maturation growth rates of offspring in experiment 2 were compared using a linear mixed effects model with size as the response variable, age as a covariate, maternal age (young, old) and clone (Boris, D8.7A, NBG70) fitted as fixed factors and individual fitted as a random term for intercept and slope. The models were implemented using the lmer function within the LME4 package version 1.7-23 (Bates et al. 2014) in R version 3.0.1. The process of maturation is stochastic rather than deterministic, such that genetically similar individuals reared under similar environmental conditions may still undergo maturation at different ages and sizes (Bernardo 1993; Morita and Morita 2002). We have previously shown that the decision to mature in *Daphnia* is best modelled using age and size intervals that precede ovary formation (stage IM-1) using a logit-link GLM, potentially with an offset (Harney et al. 2012). Consequently, we used the same approach here. Initially, models were fitted that contained clone (Boris, D8.7A, NBG70) and maternal age line (old, young) as fixed factors, age and size as covariates and the interactions between these variables. Food was not included as a factor; because in order to effectively fit PMRNs, individuals must mature at a range of ages and sizes (Heino et al 2002), and rather than being a treatment, different food levels therefore serve to generate a range of growth trajectories. Maturation rates are then integrated over size or age or both (see Harney et al. 2012). In order to find the model that best fitted the data, a large number of different GLMs (57 in total) were fitted simultaneously, and the model with the lowest AIC was selected. In different models, age and size were fitted as covariates singly (age or size) and in combination (age and size), using interval start-, mid- or endpoints, and with either untransformed or log-transformed values, and GLMs were fitted with and without offsets for intervals, with both age offsets and size offsets considered (see Harney et al. 2012 for more details). Once the model with the lowest AIC had been chosen from among these 57 possible models, the importance of clone, maternal age and their interactions with each other, and with age and size, was determined using likelihood ratio tests. PMRNs were visualised by simulating growth curves for the best fitting model and calculating maturation probabilities per curve, then approximating and plotting the 25th, 50th and 75th percentiles (see Van Dooren et al. 2005 for details). All statistical analyses and generation of PMRNs were conducted in R (version 2.13.2) using packages Hmisc (Harrell 2012a), gplots (Warnes 2012), lme4 (Bates et al. 2014), MASS (Venables and Ripley 2002), survival (Therneau 2013), rms (Harrell 2012b) and arm (Gelman and Yu-sung 2014).

**RESULTS**

Experiment 1

*The effect of clone and maternal age line on offspring life history and lifespan*.

The life histories of 4th generation offspring differed between clones (MANOVA, *Pillai's trace* = 1.051, *F*2,74 = 10.938, P<0.0001). But the non-genetic inheritance effect associated with maternal age line was even stronger (MANOVA, *Pillai's trace* = 0.825, *F*2,74 = 45.728, P < 0.0001) and varied between clones (MANOVA, *Pillai's trace* =1.432, *F*2,72 = 24.149, P < 0.0001). In the PCA, 48% of the total offspring phenotypic variation was explained by PC1, which was closely aligned with maternal age line (R2 = 0.7, P < 0.0001, see Fig 2A-C). Offspring from the old maternal line were larger neonates than those from the young maternal line, with higher growth rates, and larger size at maturation but they also had reduced lifespan and lifetime reproductive success (Fig. 2B,C). PC2, which explained 25.4% of the total life-history variation, was predominantly associated with offspring from the old maternal age taking longer to mature in clone Boris. The pairwise comparisons of the phenotypic change vectors revealed that maternal age line effects were of the same magnitude in clones Boris and D8.7A, but significantly reduced for clone NBG70. In contrast, the angle of the phenotypic change vector differed for all 3 clones demonstrating that responses to maternal age line were all clone specific (see Fig 2D).

*The effect of clone and maternal age line on offspring age-specific reproduction*

The GAMM analysis revealed considerable age-dependent variation in clutch sizes across lifetimes (model 3 vs model 2, Table 1, ΔAIC=180.4). For all clones together, clutch sizes increased until around day 20 - 30, after which there was clear evidence of reproductive senescence (see Fig. 3A). Offspring from the old maternal age line had significantly increased clutch sizes earlier on in life (model 5 vs model 4, Table 1, ΔAIC=167.2) and subsequently demonstrated a much earlier onset of senescence (23 days old) compared to offspring from the young maternal age line (34 days old, see Fig. 3A). Once senescence had begun, there was no difference in the rate of reproductive decline in offspring from young and old maternal lines (LRT, df = 1, χ2 = 1.80, P = 0.18). Adding clone specific smoothing functions improved the fit of the model (model 9, Table 1). All clones showed a similar pattern, with offspring from the old maternal line demonstrating an earlier onset of senescence (see Fig. 3B-D).

*The effect of genotype and maternal age ancestry on offspring age-specific mortality rates*

Based on AIC scores, the Gompertz survival distribution where survival depended on clone and maternal line gave the best fit to the data, though there was some support for the Weibull distribution (ΔAICc=2.27). The data did not support an Exponential distribution (ΔAICc=165.78), showing that the mortality rates did increase significantly with age in this study (LRT, df=3, χ2 = 181.6, P < 0.001). The remaining results assumed a Gompertz survival distribution, though we found the same qualitative conclusions when using a Weibull distribution.

Survival depended significantly on clone (LRT, df=8, χ2 = 66.67, p < 0.001) and on maternal age line (LRT, df=6, χ2 = 81.26, p < 0.001), but the effect of maternal age differed between clones (Figure 4). Age-specific mortality accelerated more quickly in the old maternal lines of clones Boris (LRT, df=2, χ2 = 37.06, p < 0.001) and D8.7A (LRT, df=2, χ2 = 38.84, p < 0.001), demonstrating a strong Lansing effect. However, age-specific mortality did not differ between maternal lines in NBG70 (LRT, df=2, χ2 = 5.36, p > 0.05).

We have included in figure 4 D-F the estimates of age-specific mortality rates obtained by binning the survival events in 3-day bins (as in Bronikowski and Flatt 2010). These are included only as a guide to the eye, as there are insufficient data points to draw any inferences from the binned data. Note that our models (illustrated by the lines in Fig. 4 D-F) were fitted directly to the survival event data, and that there is indeed enough data to test our hypotheses (as illustrated by the statistics quoted in the previous two paragraphs).

Experiment 2

*The effect of genotype and maternal age on offspring growth rates and maturation decisions.*

As in experiment 1, offspring from older mothers produced significantly larger neonates. However the effect of maternal age varied between clones (LRT: maternal age x clone, df = 2, χ2 = 555.18, P < 0.0001; Fig. 5A-C). These offspring also grew faster than the offspring produced from young mothers (LRT: maternal age x age, df = 1, χ2 = 384.26, P < 0.0001) to the extent that in all three clones, offspring from old mothers on low food grew as fast as offspring from young mothers on high food (Fig. 5A-C). This result clearly demonstrates that young mothers constrain the growth rates of their offspring. Interestingly, there was no difference in the magnitude of this effect across the different clones (LRT: maternal age x clone x age, df = 2, χ2 = 4.644, P = 0.098; Fig. 5A-C). However, there was a significant difference in the way the growth rate of the different clones responded to the food treatment (LRT: food x clone x age, df = 2, χ2 = 549.22, P < 0.0001; Fig. 5A-C).

For the analysis of maturation decisions, the GLM with the lowest AIC included a size offset and featured age and size covariates based on interval endpoints (a table of all models is included in the electronic supplementary material as Online Appendix A). The minimum adequate model did not feature interactions between maternal age and offspring age (LRT: df = 1, χ2 = 2.206, *P* = 0.1373) or size (LRT: df = 1, χ2 =1.812, *P* = 0.1781), suggesting that maternal age doesn't alter the rate at which incremental increases in size or age influence the probability of maturing. However, there was a significant maternal age by clone effect (LRT: df = 2, χ2 = 7.5023, *P* = 0.0234) caused by the fact that offspring from older mothers demonstrated a significant upward shift in the size at which maturation was initiated in clones Boris and D8.7A, but not in clone NBG70 (Fig. 5D-F).

**DISCUSSION**

Understanding how genetic and non-genetic inheritance mechanisms interact to shape trait variation may be crucial for understanding how traits evolve (Danchin 2013; Day and Bonduriansky 2011; Hallsson et al. 2012; Townley and Ezard 2013). With respect to the evolution of senescence, the role that non-genetic inheritance plays has been understudied (Priest et al. 2002). We show here that in *D. pulex*, maternal age line is the main factor contributing to a principal component that explained 48% of the total offspring phenotypic variation including substantial reductions in the lifespan of two of the three clones we studied. Our results support previous studies of the Lansing effect (Beardmore and Shami 1985; Fox et al. 2003; Gillespie et al. 2013; Hercus and Hoffmann 2000a; Jennings and Lynch 1928; King 1983; Lansing 1947; Lansing 1948; Lansing 1954; Lints and Hoste 1974; Lints and Hoste 1977; Murphy and Davidoff 1972; Priest et al. 2002; Tarín et al. 2005) and studies linking parental age effects to offspring viability (Benton et al. 2008; Fox et al. 2003; Groothuis et al. 2005; Hercus and Hoffmann 2000b; Kern et al. 2001; McIntyre and Gooding 2000; Priest et al. 2002; Reid et al. 2010; Tarín et al. 2005). Moreover, they support the hypothesis that non-genetic inheritance is an integral part of offspring trait evolution (Badyaev and Uller 2009; Danchin 2013; Priest et al. 2002).

The senescent parent hypothesis predicted that older mothers would produce offspring with an elevated ‘biological age’ meaning that they would have lower age-independent reproductive potential, lower growth rates and intrinsically higher age-independent mortality rates (frailty). Yet in experiment 1 there was no suggestion that offspring from older mothers had higher age-independent mortality rates (frailty) as might have been expected if older senescent mothers simply transmitted their senescent state to their offspring (Diaz and Esponda 2004). Instead, our results suggest that, in *D.* pulex at least, the Lansing effect is best explained by the offspring response hypothesis. Offspring from older ancestors were larger neonates that grew faster, initiated maturation at the same, or larger, body sizes (Fig. 5D-F) and demonstrated increased fecundity over the first few clutches laid (Fig. 3). This resulted in advanced reproductive senescence (Fig. 3) and an increased mortality rate (Fig. 4), explaining why offspring from older ancestors typically died sooner and had lower lifetime reproductive success. Such effects could conceivably also have come from clonal selection of *de novo* mutations. However, we think this is unlikely given that all experiments started from a single individual and the rates of trait divergence across the four generations of maternal age selection (see Table S3) were much higher than those expected for divergence based on mutations alone (Lynch et al. 1998). Clonal selection of epimutations or somatic variants is another possibility. But again, it seems unlikely as there was little differential mortality between treatments during the set-up of the experiment (see Table S2) and the response to maternal age line selection was broadly comparable in all three of the clones, despite setting up each generation with randomly selected offspring from multiple mothers.

Our findings concur with Lansing's original studies (1947; 1948; 1954) which also demonstrated earlier and increased reproduction in offspring from old maternal lines (King 1983). Moreover, Bouwhuis *et al.* (2010) recently found that in Great tits, offspring hatched from older mothers initially recruited more offspring, but then suffered from advanced and increased rates of reproductive senescence. Finally, in *Drosophila*, offspring born from older mothers showed high early life fertility but reduced late life fertility (Priest et al. 2008). However, in these previous studies the reason that offspring from older parents increased early life reproductive effort or senesced at a faster rate was unclear. The results presented in this study make the link between parental age, offspring development, life-history and rates of aging explicit. In *D. pulex*, the Lansing effect is the result of offspring responses to increased egg provisioning by older mothers. The increased provisioning leads to higher offspring growth rates. In fact, in experiment 2, offspring from old mothers (clutch 11) that were fed low food were able to grow as fast as offspring from young mothers (clutch 1) that were fed high food (Fig. 5A-C). Higher growth rates have been linked to senescence-related processes such as antioxidant defences (Blount et al. 2003), telomere dynamics (Hall et al. 2004; Houben et al. 2008; Monaghan and Haussmann 2006) and stress responses (McEwen 2007). We also found that the probabilistic maturation reaction norms (PMRN’s) of offspring from the old maternal lines were shifted upwards in two of the three clones we studied, meaning that those offspring initiated maturation at larger body sizes (Fig. 5D-E). While it is known that PMRN’s in *Daphnia* are plastic and clonally variable (Harney et al. 2012), this is the first study demonstrating that the position of a PMRN can be altered by a parental effect. Maturing at a larger size might increase mortality rates of offspring by facilitating increased early-life reproductive effort and a higher ‘cost of reproduction’ (reviewed in Roff 1992; Roff 2007; Stearns 1992), or by increasing maintenance costs that scale with body size, such as the cost of moulting (Hessen and Alstad Rukke 2000). Interestingly, the clone that showed no maternal-age related upshift in the PMRN of its offspring (see Fig. 5F) also showed no increase in the age-specific mortality rate of offspring from older mothers (see Fig. 4E) and no Lansing effect. The variable maternal age effects on offspring in different clones (see Fig. 2A) mirror genetically variable parental age effects previously observed in *Drosophila* juvenile survival (Kern et al. 2001), and *Drosophila* lifespan and age-specific mortality rates (Priest et al. 2002). Our results suggest that such variation might arise from genetic differences among mothers in their age-dependent reproductive investment and provisioning of individual offspring (see Plaistow et al. 2007). Alternatively, they could reflect differences in the way that different genotypes respond to the maternal developmental environment. Irrespective of the mechanism, our results suggest that genetic and non-genetic effects determine the life histories of offspring.

The nature of inheritance underpinning trait variation in any population is important because it may greatly alter the response of the trait to selection (Bonduriansky et al. 2011; Danchin et al. 2011; Hallsson et al. 2012). Non-genetic inheritance is especially likely to influence the evolution of environmentally sensitive traits since non-genetic inheritance can be considered an environmental component from the perspective of the genotype. Our results support studies of lifespan extension, genetic intervention and dietary restriction in other model organisms that have also revealed that senescence is a plastic trait (Flatt and Heyland 2011; Flatt and Schmidt 2009). However, they also suggest that the significance that non-genetic inheritance has on trait evolution is itself genetically variable. Given the potentially significant role that non-genetic inheritance may play in facilitating rapid evolution (Bonduriansky et al. 2011; Bonduriansky and Day 2009; Danchin 2013) this is an important result. Quantifying the extent that non-genetic inheritance differs within and between populations is an important goal for future studies.

Strong parental age effects on offspring life histories also have important ecological implications. Our results suggest that the offspring from young and old mothers are demographically very different. Parental age effects on demography and population dynamics have rarely been studied. But in the soil mite, *Sancassania berlesei* experimentally induced maternal age effects altered population dynamics for at least three generations (Benton et al. 2008). In harvested populations, such as fisheries, in which age structures are often massively truncated (Conover and Munch 2002), maternal age effects could help to explain why population dynamics are often unstable compared to non-harvested populations (Anderson et al. 2008) and some stocks are slow to recover even when fishing pressure is released (Hutchings 2000; Walsh et al. 2006).

In conclusion, we have demonstrated that in *Daphnia pulex*, genetic and non-genetic factors shape patterns of offspring aging. Older parents do not just transmit a senescent state to their offspring, as is sometimes assumed. Instead, they produce larger offspring that shift their development in a manner that increases early life-reproductive performance, resulting in advanced and increased rates of senescence that shorten offspring lifespan. Such an effect may have evolved because *Daphnia pulex* are strongly selected to breed early (they are indeterminate growers), resulting in small, young, mothers can only produce large clutches by sacrificing offspring size (Glazier 1992). This constrains the early life reproductive effort of individual offspring from young mothers, but maximises maternal fitness because she produces more offspring (Einum and Fleming 2000; Marshall and Uller 2007). The constraint that young mothers place upon their offspring may then extend offspring lifespan in a similar way that dietary restriction has been shown to extend lifespan in a vast array of different taxa (Masoro 2005; Partridge et al. 2005).

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**APPENDIX**

**Table A1** – The number of females that died prior to set-up as a fraction of the total during each generation of experiments 1 & 2 for clones Boris, D8.7A and NBG70. Occasions where there was mortality are shown in bold.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **EXPERIMENT 1** | | | | |
| **Generation** | **Clone** | **Clutch** | | |
| **Parental** |  | **1** | **3** | **5** |
|  | Boris | 0/3 |  | 0/3 |
|  | D8.7A | 0/3 |  | 0/3 |
|  | NBG70 | 0/3 |  | 0/3 |
| F1 |  |  |  |  |
|  | Boris | 0/3 |  | 0/3 |
|  | D8.7A | 0/3 |  | 0/3 |
|  | NBG70 | 0/3 |  | 0/3 |
| F2 |  |  |  |  |
|  | Boris | 0/3 |  | 0/3 |
|  | D8.7A | 0/3 |  | 0/3 |
|  | NBG70 | 0/3 |  | **2/3** |
| F3 |  |  |  |  |
|  | Boris | **2/6** |  | 0/6 |
|  | D8.7A | **1/6** |  | 0/6 |
|  | NBG70 | 0/6 |  | 0/6 |
| **EXPERIMENT 2** | | | | |
| Parental |  | 1 | 3 | 5 |
|  | Boris |  | 0/3 |  |
|  | D8.7A |  | 0/3 |  |
|  | NBG70 |  | 0/3 |  |
| F1 |  |  |  |  |
|  | Boris |  | 0/3 |  |
|  | D8.7A |  | 0/3 |  |
|  | NBG70 |  | 0/3 |  |
| F2 |  |  |  |  |
|  | Boris |  | **1/3** |  |
|  | D8.7A |  | 0/3 |  |
|  | NBG70 |  | 0/3 |  |
| F3 |  |  |  |  |
|  | Boris |  | **1/8** |  |
|  | D8.7A |  | **4/8** |  |
|  | NBG70 |  | **3/8** |  |

**Table A2** – The mean divergence of traits in 4th generation offspring from experiment 1 as a percentage of the overall trait mean. The rate of trait divergence is on average 10-fold higher than that expected from mutation alone (values taken from Lynch, 1998 following 32 generations of mutation accumulation and intentional clonal selection).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Trait** | **Clone** | **Overall mean** | **Old**  **maternal**  **age line** | **Young**  **maternal**  **age line** | **% trait divergence** | **% trait divergence from mutation (from Lynch 1998)** |
|  | Boris | 0.632 | 0.665 | 0.611 | 8.563 |  |
| **Neonate length** | D8.7A | 0.682 | 0.714 | 0.634 | 11.709 | 0.5% |
|  | NBG70 | 0.665 | 0.707 | 0.623 | 12.635 |  |
|  | Boris | 0.170 | 0.193 | 0.155 | 22.069 |  |
| **Growth (intercept)** | D8.7A | 0.208 | 0.236 | 0.167 | 33.315 | N/A |
|  | NBG70 | 0.203 | 0.211 | 0.194 | 8.064 |  |
|  | Boris | 0.871 | 0.821 | 0.902 | 9.269 |  |
| **Growth (slope)** | D8.7A | 0.852 | 0.852 | 0.853 | 0.118 | N/A |
|  | NBG70 | 0.835 | 0.820 | 0.850 | 3.632 |  |
|  | Boris | 1.815 | 1.961 | 1.721 | 13.192 |  |
| **Size at maturity** | D8.7A | 1.805 | 1.909 | 1.649 | 14.384 | 0.8% |
|  | NBG70 | 1.784 | 1.820 | 1.747 | 4.059 |  |
|  | Boris | 11.087 | 12.444 | 10.214 | 20.115 |  |
| **Age at maturity** | D8.7A | 6.240 | 5.000 | 8.100 | 49.679 | 3% |
|  | NBG70 | 7.133 | 7.267 | 7.000 | 3.738 |  |
|  | Boris | 95.783 | 29.333 | 138.500 | 113.973 | 3% |
| **Lifetime fecundity** | D8.7A  NBG70 | 100.480  118.667 | 83.667  117.333 | 125.700  120.000 | 41.833  2.247 | (average for  clutches 1-4) |
|  | Boris | 33.304 | 19.556 | 42.143 | 67.821 | 0.8% |
| **Survival** | D8.7A | 30.080 | 25.400 | 37.100 | 38.896 | (until |
|  | NBG70 | 29.900 | 29.333 | 30.467 | 3.790 | maturity) |
|  |  |  |  | **Mean=** | **23.005 %** | **1.52%** |

**Table A3** - Mean values and univariate GLM’s for all traits used in the MANOVA in experiment 1.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Trait** | **Mean for young maternal age line** | **Mean for old maternal age line** | **Results of individual GLM’s** | | |
|  |  |  | **Clone** | **Treatment** | **Clone x Treatment** |
| Neonate | 0.611 | 0.665 | F2,72=16.84\*\*\* | F1,72=278.38\*\*\* | F2,72=3.62\* |
| 0.634 | 0.714 |
| 0.623 | 0.707 |
| Growth (intercept) | -0.023 | 0.281 | F2,72=13.19\*\*\* | F1,72=139.45\*\*\* | F2,72=15.73\*\*\* |
| 0.141 | 0.217 |
| 0.131 | 0.298 |
| Growth (slope) | -0.001 | -0.017 | F2,72=64.99\*\*\* | F1,72=43.86\*\*\* | F2,72=63.63\*\*\* |
| 0.003 | 0.085 |
| 0.029 | 0.021 |
| Size at maturity | 1.721 | 1.961 | F2,72=2.41NS | F1,72=72.15\*\*\* | F2,72=8.85\*\*\* |
| 1.649 | 1.909 |
| 1.747 | 1.820 |
| Age at maturity | 10.214 | 12.444 | F2,72=84.52\*\*\* | F1,72=7.71\*\* | F2,72=23.31\*\*\* |
| 8.100 | 5.000 |
| 7.000 | 7.267 |
| Fecundity | 138.500 | 29.333 | F2,72=2.91NS | F1,72=21.81\*\*\* | F2,72=10.40\*\*\* |
| 125.700 | 83.667 |
| 120.000 | 117.333 |
| Survival | 42.143 | 19.556 | F2,72=0.67NS | F1,72=45.78\*\*\* | F2,72=14.77\*\*\* |
| 37.100 | 25.400 |
| 30.467 | 29.333 |

**DATA ACCESSIBILITY**

The data sets supporting this article are deposited in the DRYAD Digital depository (Plaistow et al. 2015)

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**Table 1.** A statistical comparison of additive mixed models in which non-parametric smoothing functions for age were fitted to age-specific clutch sizes for all individuals, and subsets of individuals, based on maternal age treatment and clone identity.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Model No.** | **Terms** | **No. functions** | **AIC** | **Model comparison** | ***F*** | ***P*** |
| 1 | R1 | 1 | 4139.3 |  |  |  |
| 2 | R1+R2 | 2 | 3875.7 | vs 1 | 10.66 | <0.0001 |
| 3 | A+ R1+R2 | 3 | 3695.3 | vs1 | 19.24 | <0.0001 |
|  |  |  |  |  |  |  |
|  | *Maternal age line effects* |  |  |  |  |  |
| 4 | T +A+ R1+R2 | 3 | 3690.6 | vs 3 | 1.31 | 0.229 |
| 5 | T+(T x A)+A+ R1+R2 | 5 | 3523.4 | vs 4 | 33.48 | <0.0001 |
| **6** | **T+(T x A)+ R1+R2** | **4** | **3523.4** | **vs5** | **2.51** | **0.008** |
|  |  |  |  |  |  |  |
|  | *Clone effects* |  |  |  |  |  |
| 7 | C+T+(T x A)+A+ R1+R2 | 4 | 3517.0 | vs 5 | 0.90 | 0.502 |
| 8 | C+(C x A)+T+(T x A)+A+R1+R2 | 8 | 3329.5 | vs 5 | 14.79 | <0.0001 |
| **9** | **C+(C x A)+T+(T x A)+R1+R2** | **7** | **3329.5** | **vs 8** | **0.59** | **0.008** |

Note: The models that best fitted the data with and without clone effects are shown in bold face. R1= random intercept term for individual; R2 = random age term for individuals; A = age term for all individuals; T = maternal age treatment (Old line, Young line) and C = the clone effect (Boris, D87A, NBG70).

**FIGURE LEGENDS**

**Figure 1A.** Shows the experimental design used for experiment 1. For each of the 3 clones used in this experiment (Boris, D8.7A, NBG70), young and old maternal age lines were created by randomly selecting offspring from young mothers (clutch 1) or old mothers (clutch 5) to set up each new generation. The effect of maternal age line on offspring life-histories, lifespan and rates of senescence was then compared in 4th generation by randomly selecting 15 offspring from the 1st clutch of females in the young maternal age line and 15 offspring from the 5th clutches of females in the old maternal age line (see methods for details). **Figure 1B.** Shows the experimental design used for experiment 2. For each of the 3 clones used (Boris, D8.7A, NBG70), offspring from middle-aged mothers (clutch 3) were conditioned on high food for three generations. The numbers in brackets refer to the number of mothers set-up in each generation. In the 4th generation, the effect that maternal age had on offspring development was tested by comparing the growth rates and maturation decisions (PMRN's) of 30 randomly selected offspring from mothers 1st clutches and 30 randomly selected offspring from the same mothers 11th clutches (see methods for details).

**Figure 2A.** The scores plot for the PCA. Individuals from the old maternal line are plotted as solid points for Boris (circles) NBG70 (squares) and D87A (triangles) whereas as individuals from the young maternal lines are plotted as clear points. The mean multivariate phenotypes of each clone after 3 generations of maternal age selection are plotted as stars and linked by solid line vectors, labelled at each end. The average effect of the maternal age selection is plotted as large stars, linked by a dashed line vector. **Figure 2B.** A vector plot of the loadings from the PCA. Vectors that are close in space indicate positive correlations between traits. Vectors that point in opposite directions are negatively correlated, and vectors that are perpendicular are uncorrelated. The length of the vector indicates the amount of variation associated with it. **Figure 2C.** Details of the variation explained by each component and the loadings. **Figure 2D.** Pairwise comparisons of differences in the length and direction of phenotypic change vectors, summarizing the differences in the multivariate phenotypes of the three clones when reared from young and old maternal age lines. Significant differences shown in bold.

**Figure 3.** Age-specific reproductive effort of offspring from young (grey dots, dashed lines) and old (black dots, solid lines and light grey shaded area) maternal lines for (A) all clones combined; and (B) clone Boris, (C) clone D8.7A and (D) clone NBG70. The lines represent the reproductive effort predicted by best-fitting generalized additive mixed models (GAMM's, see Table 1) for offspring from the old maternal line (solid lines) and the young maternal age line (dashed lines). The age at the onset of reproductive senescence was predicted as the peak of each fitted GAMM and is marked as a thin dashed line. In all cases, early clutch sizes were larger, and the age at the onset of reproductive senescence was earlier, for offspring from the old maternal age line. However, there was no difference in the rate of reproductive senescence between maternal age line treatments.

**Figure 4.** The survival probability (A-C) and log mortality rate (D-F) of offspring from the old maternal age line (solid lines) and the young maternal age line (dashed lines), for the three different clones (panels A & D: Boris; panels B & E: D8.7A; panels C & F: NBG70). The thick lines are the predictions from a best fitting parametric survival model with a Gompertz error distribution, with 95% confidence intervals in the predicted survival as grey shaded regions. For comparison purposes, thin lines show Kaplan-Meijer estimates of the survival rate and points (triangles: old maternal line; circles: young maternal line) show the estimated hazard obtained by binning the events in 3-day bins.

**Figure 5**. The top panels show the effect of maternal age and food on the growth rates of offspring from clones (A) Boris; (B) D8.7A; and (C) NBG70. Solid lines and points represent the growth rates of offspring fed on high food, whereas dashed lines and clear points show offspring fed on low food. Offspring from the old maternal age line are shown as black lines and points whereas offspring from the young maternal age lines are show as grey lines and points. The bottom panels show the effect of maternal age on the probabilistic maturation reaction norms of offspring from clones (D) Boris; (E) D8.7A; and (F) NBG70. Dark grey lines culminating in black circles represent growth and maturity of old (clutch 11) mothers, and are intersected by black 25, 50 and 75% PMRN's from the best fitting model. Pale grey lines represent growth trajectories of offspring born to young (clutch 1) mothers, culminating in pale grey circles at maturity. These growth trajectories are intersected by grey 25, 50 and 75% PMRNs.

Fig. 1

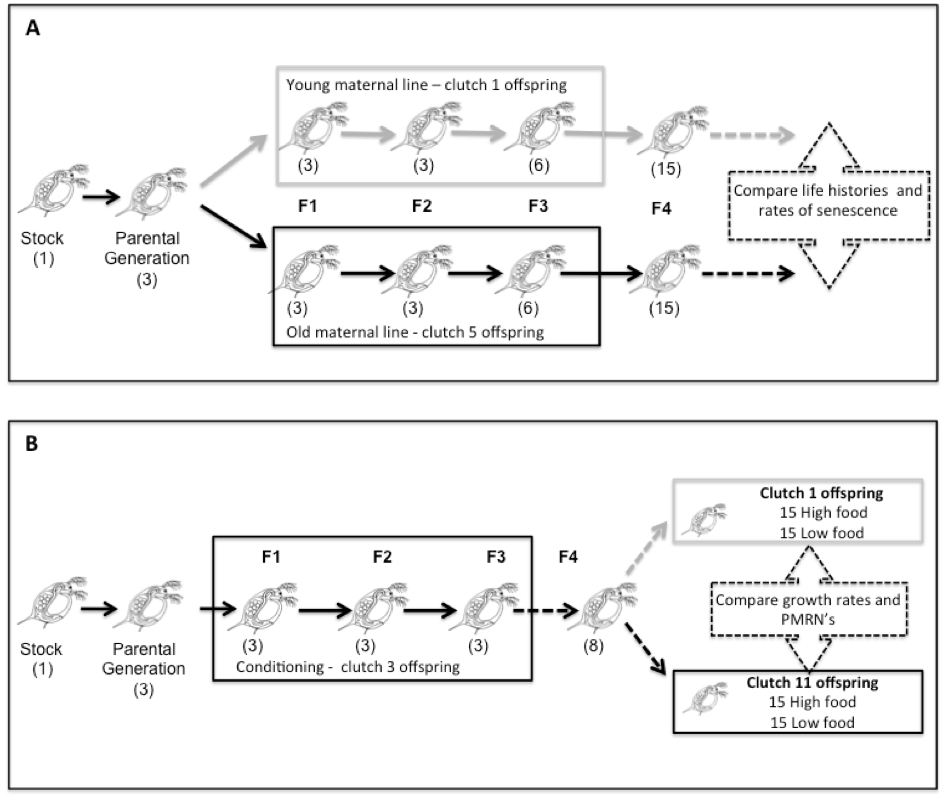


Fig. 2



Fig. 3

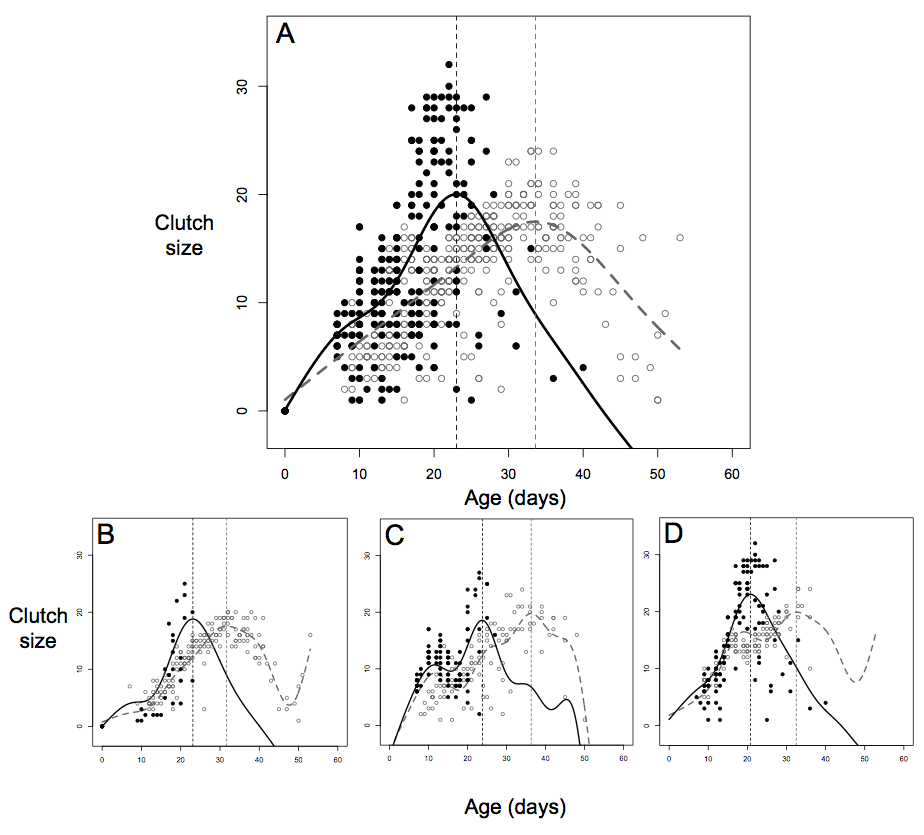


Fig. 4

Fig. 5

