

# Maternal effects mediated by maternal age: from life histories to population dynamics

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## Summary

1. Maternal effects describe how mothers influence offspring life histories. In many taxa, maternal effects arise by differential resource allocation to young, often identified by variation in propagule size, and which affects individual traits and population dynamics.

2. Using a laboratory model system, the soil mite *Sancassania berlesei*, we show that, controlling for egg size, older mothers lay eggs that hatch later, develop more slowly, and mature at larger body sizes.

3. Such differences in life histories lead to marked population dynamical effects lasting for multiple generations, as evidenced by an experiment initiated with similarly sized eggs that came from young or old mothers. Differences in maturation from the initial cohort led to differences in population structure and life history that propagated the initial differences over time.

4. Maternal-age effects, which are not related to gross provisioning of the egg and are therefore phenotypically cryptic, can have profound implications for population dynamics, especially if environmental variation can affect the age structure of the adult population.

**Key-words:** density dependence, intergenerational effects, reproductive allocation, senescence, transient population dynamics

Understanding the causes of phenotypic variation between individuals is necessary to be able to predict how populations may respond to environmental change and is also important for understanding the interplay between evolutionary and ecological dynamics (Benton *et al.* 2006; Coulson *et al.* 2006; Pelletier *et al.* 2007). Individuals obviously vary because they contain different genes, or they live in different environments and therefore, for example, have access to different resources. However, there is also increasing evidence from many taxa that parents influence offspring life histories through parental effects (for a recent review see Räsänen & Kruuk 2007 and references therein).

Parental effects occur when variation in parental phenotype, which may be caused genetically or in response to the environment the parent inhabits, affects offspring phenotype. Parental effects can arise through a number of routes. First, parental genotypes can affect patterns of offspring development. For example, the maternally expressed *CLK* (for CLOCK) genes

in *Caenorhabditis elegans* affect offspring patterns of development, growth rate, fecundity, behaviour and survival (Branicky, Benard & Hekimi 2000; Benard *et al.* 2001). Second, parental effects can arise from differential allocation of resources to the offspring and this variation is typically associated with parental condition and/or environment. Such resource-related maternal effects can have profound implications for offspring growth, development and other traits, such that cohorts of animals, born under different environmental conditions, may carry the signature of the maternal environment through life (Lindstrom 1999; Forchhammer *et al.* 2001; Beckerman *et al.* 2003). By changing offspring phenotype, these resource-related parental effects may affect the ability of offspring to provision the next generation, effectively leading to grand-parental effects (Drake, Walker & Seckl 2005; Plaistow, Lapsley & Benton 2006). Maternal provisioning need not solely be of nutritional benefit. For example, maternally produced antibodies may be passed onto offspring, thereby facilitating the offspring's response to immune challenge (Gasparini *et al.* 2006; Martinez-Padilla 2006); or the maternally-invested titre of steroid hormones in avian eggs can influence offspring behaviour, growth trajectory, morphology, immunology and survival, with some effects being detectable long after the offspring have fledged (Groothuis *et al.* 2005). Similarly,

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juvenile development can be 'programmed' by maternal changes in the egg or developmental environment (Zhang *et al.* 2006). Third, parental effects can arise indirectly through alteration of the offspring environment. This may occur when fecundity and per-capita offspring provisioning are negatively related. By changing per-capita provisioning, parents may also change their own fecundity, leading to changes in the competitive environment faced by the offspring. This fecundity-mediated change in competitive environment may have a much stronger impact on offspring life histories than altering per-egg provisioning strategies (Beckerman *et al.* 2006). The presence of these indirect, density-dependent effects, coupled with the context dependence of 'egg quality' effects (in that, for example, a larger egg size may influence different offspring traits depending on the environment in which the offspring are born: Plaistow *et al.* 2006) suggests that greater attention should be paid to investigating parental effects in an ecological setting.

Several recent studies have highlighted the fact that juvenile survival can change in response to maternal age (e.g. Berkeley, Chapman & Sogard 2004; Paitz *et al.* 2007; Zehnder, Parris & Hunter 2007). As mothers age, their egg provisioning may change in response to changes in the trade-off between optimal investment in maintenance and reproduction (e.g. Pianka & Parker 1975) or in response to changes in the environment. For example, Plaistow *et al.* (2007) showed that in soil mites, females switched from laying many small eggs when they were young to laying larger eggs as they got older. This pattern might be adaptive if it increases the survival of younger offspring who must compete with their older, larger siblings (Plaistow *et al.* 2007). Variation in the content of the egg (whether nutritional or some other quantity like hormone titre) may also occur independently of the gross size of the egg. For example, Giron & Casas (2003) showed that egg size changes with a mother's age, but also that the nutrient composition of eggs may vary independent of size. Thus, maternal influences may be cryptic, in that they may affect aspects of the embryonic environment which are hard to measure from examination of the size of the propagule.

Using the easily manipulated laboratory model organism, the soil mite *Sancassania berlesei* (Michael), we have previously shown that mothers alter the size of eggs as they age (Benton *et al.* 2005; Plaistow *et al.* 2007), and differences in egg size between founding cohorts can have important consequences for transient population dynamics. Our aim in this study is to investigate whether, after controlling for egg size, mothers' age influences offspring performance, and if so whether the consequence for individuals and/or transient population dynamics is of comparable magnitude to the known effects of variation in egg size. If maternal age has an effect independent of egg size, then two cohorts of eggs of similar size can initially appear phenotypically identical but can result in different demographic patterns and therefore result in different population dynamics. Without knowledge of maternal age, the difference in population performance between cohorts may appear 'random', whereas in principle it is predictable.

## Materials and methods

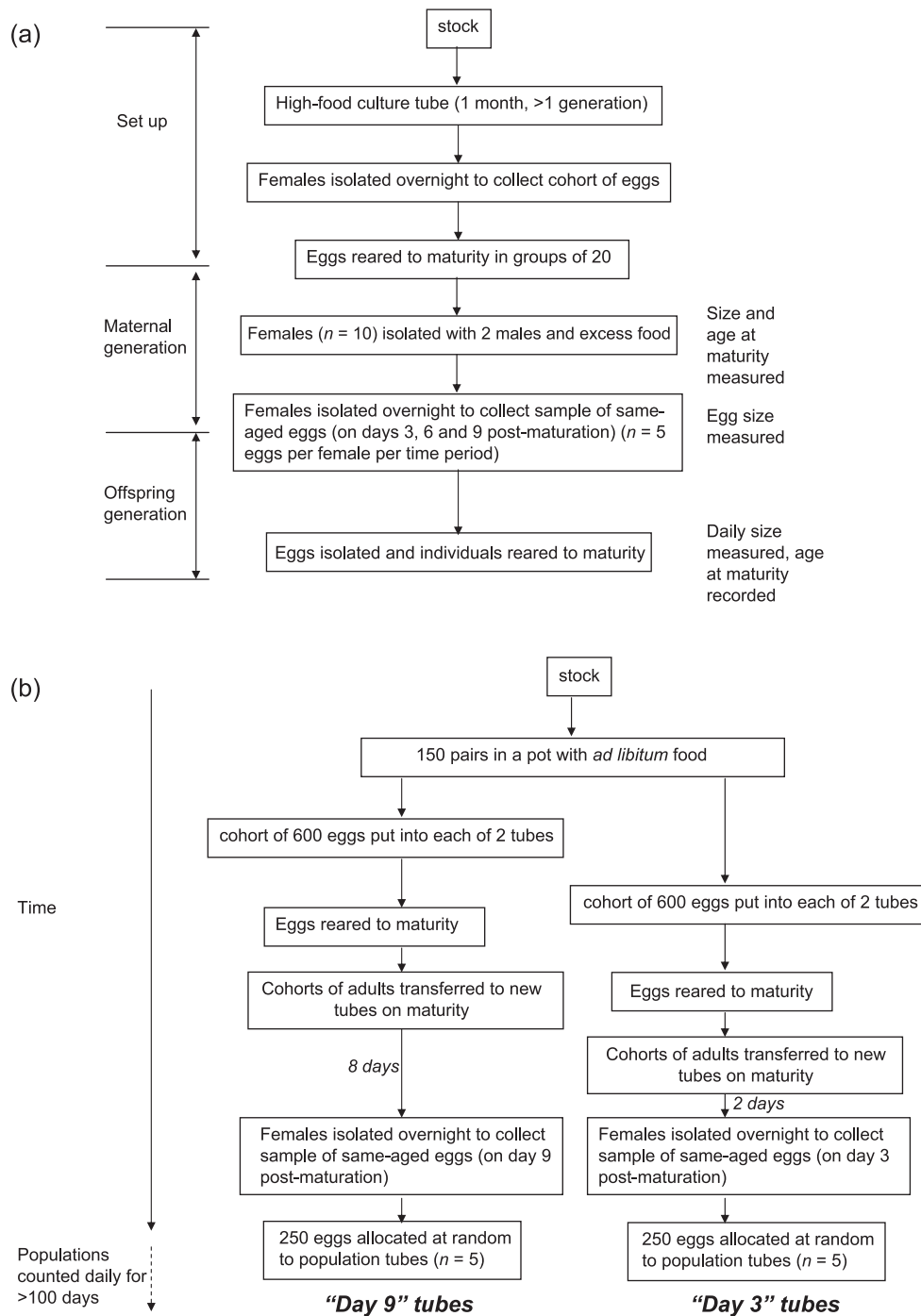
To investigate the maternal-age effect, we conducted linked experiments at two scales: one at the scale of the individual and one at the scale of the population. Both scales were necessary as it is not possible to extrapolate from experiments on individuals to population performance because individuals in populations are subject to very different environments than individuals in isolation and are likely to respond very differently. For the individual experiments, we examined the effect of maternal age on offspring performance by investigating how juvenile characteristics changed as their mothers aged, and through statistical analysis we then partitioned the variation in offspring performance between variation in egg size and variation in maternal age. For the population experiment, we conducted a population dynamical experiment to assess the differences in transient population dynamics that arose from cohorts of eggs of similar sizes but that came from mothers of different ages. The first experiment tested whether maternal age affects offspring traits independently of egg size, whereas the second established whether such effects on individuals can lead to population-level consequences.

For basic culturing procedures, see elsewhere (Benton & Beckerman 2005).

### MATERNAL EFFECTS MEASURED AT THE SCALE OF THE INDIVIDUAL

All mites used in this assay came from a stock culture originating from soil samples collected in North Yorkshire in September 2005, which were then kept under continuous laboratory culture for > 10 generations. Mites from this culture were used to set up cultures with standard conditions to minimize the potential for non-experimental effects. These, well-fed, low density standard cultures each contained approximately 50 adults (see Fig. 1a for schematic of design). Females collected from a single well-fed culture were isolated for 24 h, and eggs laid during this period were collected and allocated to five rearing tubes (23 mm in diameter) at a density of 20 eggs per tube. When the first 10 larvae had emerged in a tube, an excess of food (yeast) was added. On maturity, a total of 15 females which had matured overnight (~15-h period) were selected at random, isolated and photographed. Each female was placed in a small (13 mm diameter) individual rearing tube and supplied with food in excess (one 1.125 mm diameter ball of dried yeast, mean  $1.12 \pm \text{SD } 0.20$  mg, every other day). Two males were also added. These males were collected as final instar nymphs from the well-fed culture, and allocated to females 2–4 days after maturity (thereby controlling for the paternal effects that may have arisen from different aged fathers). Two males were used to avoid possible effects of sperm depletion or sterility. Eggs were collected by moving each female into a clean tube with two newly matured males in the evening and removing her in the morning of the 2nd, 5th and 8th nights following her moult to maturity. Five eggs were selected from each of 10 mothers at each age, and allocated individually to small rearing tubes (giving, in total, 150 eggs: 10 mothers  $\times$  3 periods  $\times$  5 eggs). One of the females died before reaching the third sampling point, and was replaced with one of five spares kept under identical conditions for that contingency. The eggs were photographed the day after they were laid, and checked daily to record hatching. On hatching, each larva was supplied with excess food. Every day each individual was fed, watered and photographed and its life history stage recorded until 2 days after maturation.

Digital images were measured using the image analysis package imagej 1.28U (<http://rsb.info.nih.gov/ij>). Lengths of nymphs and



**Fig. 1.** Schematics of experimental design. (a) Experiment 1, used to assess the magnitude of maternal effects on offspring performance; (b) Experiment 2, used to investigate the way the maternal age of a founding cohort of eggs causes different population dynamical trajectories.

adults were measured from the tip of the hypostome to the tip of the opisthosoma. Egg volumes were estimated as the volume of a prolate spheroid:  $volume = 4/3 \cdot \pi \cdot [(length/2) \cdot (breadth^2/4)]$ .

To determine the effect of maternal age on egg size, egg volume, and size at maturity, we fitted linear mixed-effect models using the NLME package in R version 2.3 (R Development Core Team 2005). Maternal identity was fitted as a random factor to correct for the non-independence of offspring from the same mother. Maternal effects on hatching and maturation were analysed using generalized

linear mixed models (GLMM) using the LMER function contained within the LME4 and MATRIX packages in R. Maternal identity was included as a random term to control for the non-independence of offspring. Models were fitted using a binomial error structure and a logit link function. We used the laplace approximation method, contained within the LMER function (LME4 package). Finally, maternal effects on development time were analysed using a parametric survival model implemented using the 'survreg' function contained within the SURVIVAL (version 2.28) package in R. Maternal identity

was fitted as a fixed effect. Minimum adequate models were derived using backwards, stepwise procedures to remove nonsignificant terms. Model selection was based upon Akaike information criterion (AIC). All models are presented with degrees of freedom and likelihood-ratio test (LRT) statistics.

#### MATERNAL-AGE EFFECTS MEASURED AT THE SCALE OF THE POPULATION

One cannot extrapolate from the individual-level experiment to the population level (or vice versa) as any change in demographic rates will change the competitive environment and access to food, which will lead to further changes in demographic rates so that disentangling the effects due to competition or maternal effect becomes complex. Here, we initiated populations with cohorts of 250 similarly sized eggs from either 'young' (3 days post-maturity) or 'old' (9 days post-maturity) mothers in order to investigate whether initial differences in demographic rates created by the maternal-age effects could lead to different transient population dynamics. For a schematic of the experimental design, see Fig. 1b. On 2 March 2004 (Day 1), two 5-cm diameter containers fitted with breathable mesh lids and filled with a plaster-of-paris base were each inoculated with 150 males and 150 females taken from stock cultures that had been maintained at equilibrium since 2002. Both containers were kept in an incubator at 24 °C and given *ad libitum* yeast until the females were producing large clumps of eggs on a daily basis. Surviving adults were transferred on Day 6 into two fresh containers. The following day, 600 eggs were collected from each tube and placed into two 10-cm diameter pots to create a cohort of adults from which to harvest the experimental eggs. The rationale for such large numbers of mites in these 'egg factories' (cf. 'egg factories' of 50 mites in Experiment 1) is that in moderate-high density cultures, egg size does not vary as much as mothers age (Plaistow *et al.* 2007). After 2 days, approximately 50% of the eggs had hatched, and each pot was provisioned with  $7 \times 1$  mm balls of yeast a day. By Day 15, approximately 50% of individuals had matured. On Day 23, the 8-day post-maturation adults were transferred to a fresh pot to ensure that all eggs collected on the following day would be laid on the same day and would have been laid by mothers that were 9 days post-maturation. On the following day, batches of 50 eggs were removed from the two pots and randomly allocated to one of five culture tubes until all tubes contained 250 eggs. Eggs from 3-day post-maturation mothers (hereafter Day 3 mothers) were obtained in an identical fashion, but the set-up procedure was initiated 6 days later such that the Day 3 mothers' populations and the 9-day post-maturation mothers (hereafter Day 9 mothers) populations could be set up synchronously.

Samples of 20 eggs from all ten population tubes were photographed at the beginning of the experiment in order to compare the size of the eggs from the Day 3 and Day 9 mothers. Photos were taken at a magnification of  $\times 15$ , using using a Canon Powershot S40 digital camera connected to a Vision Engineering 'Lynx' head-up stereomicroscope (Vision Engineering Ltd., Woking, UK). Each day, all tubes received two drops of water and a single granule of active dried yeast, sieved for conformance ( $1.08 \text{ mg} \pm \text{s.e. } 0.03$ ,  $n = 100$ ). We counted the numbers of eggs, juveniles and adults under a Leica MZ8 (Wetzlar, Germany) binocular microscope using a hand-held tally counter, and a sampling grid scratched onto the plaster base of the tube. All adults, juveniles and eggs were initially counted throughout the tube, but after numbers exceeded 500, the juveniles and eggs were counted from a randomly selected quarter of the tube.

Analyses of the time series was undertaken with resampling methods, as, due to temporal changes in the time series' autocovariance

structure, fitting standard autoregressive models to the data is not appropriate (as, with density-dependent generation time, there is no fixed lag in the dynamics). Instead, significant divergence between time series was assessed by estimating the bootstrap confidence intervals for mean differences between the five replicates at each time point (using *R*). In cases where the confidence intervals did not overlap zero, we assumed that the time series are different. Qualitatively similar conclusions arise whether one assesses significance from bootstrapping the treatment means or by the inspection of whether the confidence intervals overlap. This latter method is slightly more conservative.

Other standard statistical tests, not described above, were conducted in *R* and normal distributional assumptions were tested.

## Results

### MATERNAL EFFECTS: INDIVIDUAL LEVEL

#### *Effects of maternal age and maternal size on egg size*

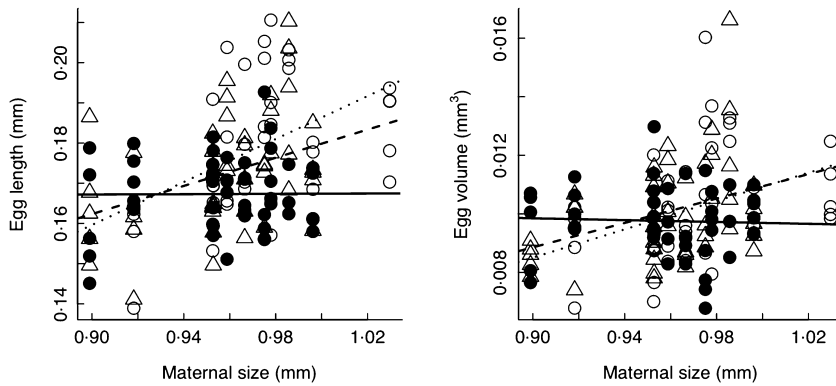
The size of young mothers did not affect the size of the eggs they laid. However, in older females, egg length increased with maternal size giving rise to a significant interaction term (d.f. = 2, LRT = 8.54,  $P = 0.014$ , Fig. 2a). When egg volume was analysed instead of egg length, the same interaction between maternal size and maternal age was seen (d.f. = 2, LRT = 6.25,  $P = 0.044$ , Fig. 2b). Given the qualitative similarity between egg size as measured by length alone, and that measured by volume, hereafter we report only results from volumetric analyses to avoid repetition.

#### *Effects of maternal age and maternal size on offspring hatching time*

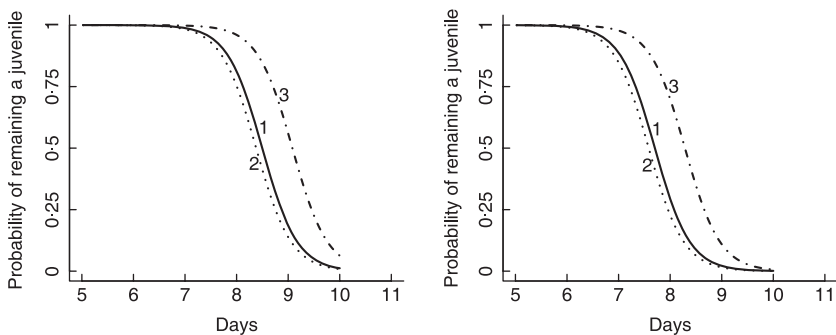
There was little variation in the time taken for eggs to hatch; of 150 eggs only three failed to hatch, and nearly all of the rest all hatched on either the 3rd or 4th day after laying ( $n = 33$ ,  $n = 113$  respectively). In order to determine the effect of maternal age on the probability of hatching on day 3, we fitted a general linear mixed model (GLMM). Eggs from older mothers were less likely to hatch on day 3 (22.8%, 32.4%, and 6.4% for ages classes 1, 2 and 3, respectively; d.f. = 2, LRT = 12.877,  $P = 0.002$ ). Egg volume had no effect on the probability of hatching on day 3, either directly (d.f. = 1 LRT < 0.01,  $P = 0.99$ ), or via an interaction with maternal age (d.f. = 2 LRT = 1.247,  $P = 0.536$ ).

#### *Effects of maternal age and maternal size on offspring development time*

Of the 150 eggs, 140 reached maturity (three eggs didn't hatch, three were known to die and four were unaccounted for as small juveniles, which probably means they died and their cadavers disintegrated without trace). Mites matured on the 8th, 9th, 10th or 11th day after laying ( $n = 73$ ,  $n = 48$ ,  $n = 18$  and  $n = 1$  respectively). To examine the effect of maternal age on development time, we fitted an accelerated failure time model using a logistic distribution (chosen via censored residual



**Fig. 2.** The relationship between egg length (a), or egg volume (b) and maternal size for 2-day-old mothers (solid circles, solid line), 5-day-old mothers (clear triangles, dashed line), and 8-day-old mothers (clear circles, dotted line). The lines represent the predictions from the best fitting linear mixed effects model.



**Fig. 3.** The probability of a juvenile maturing depends upon the egg size it hatched from and maternal age when the egg was laid. Lines are predictions from the best fitting parametric survival model (see text), with codes for 2-day-old mothers (1), 5-day-old mothers (2), and 8-day-old mothers (3). The left hand panel show predictions for 'small' eggs (egg vol = 0.0067), the right hand panel for 'large' eggs (egg vol = 0.0167).

diagnostics (Harrell 2006)). Development time was not influenced by offspring sex (d.f. = 1, LRT = 2.538,  $P = 0.111$ ). In general, controlling for egg volume, older mothers produced offspring that took longer to reach maturity (d.f. = 2, LRT = 27.96,  $P < 0.001$ , Fig. 3), whereas individuals hatching from larger eggs had shorter development times (d.f. = 1, LRT = 5.036,  $P < 0.025$ , Fig. 3a vs. 3b). Irrespective of maternal age, and the size of eggs laid, maternal identity also significantly influenced offspring development rates, with the fastest developing broods maturing up to 1.5 days earlier than the slowest under the same conditions (d.f. = 10, LRT = 22.953,  $P = 0.011$ ). Given females were reared under standard conditions, this maternal identity effect is likely to have a strong genetic component.

#### Effects of maternal age and maternal size on offspring size at maturity

Offspring hatching from larger volume eggs matured at larger body sizes (d.f. = 1, LRT = 4.259,  $P < 0.039$ , Fig. 4). However, body size was also dependent upon an interaction between the sex of the offspring and the age of the mother that laid the egg (d.f. = 2, LRT = 11.443,  $P = 0.003$ ). The offspring's size at maturation increased with maternal age in both sexes, but the effect was stronger in females who matured at a larger body size (Fig. 4a,b). A similar analysis, modelling the probability of maturing at a given size, indicates that large egg volume increased the probability of the offspring maturing at larger sizes (d.f. = 1, LRT = 5.77,  $P = 0.016$ ), but the mother's

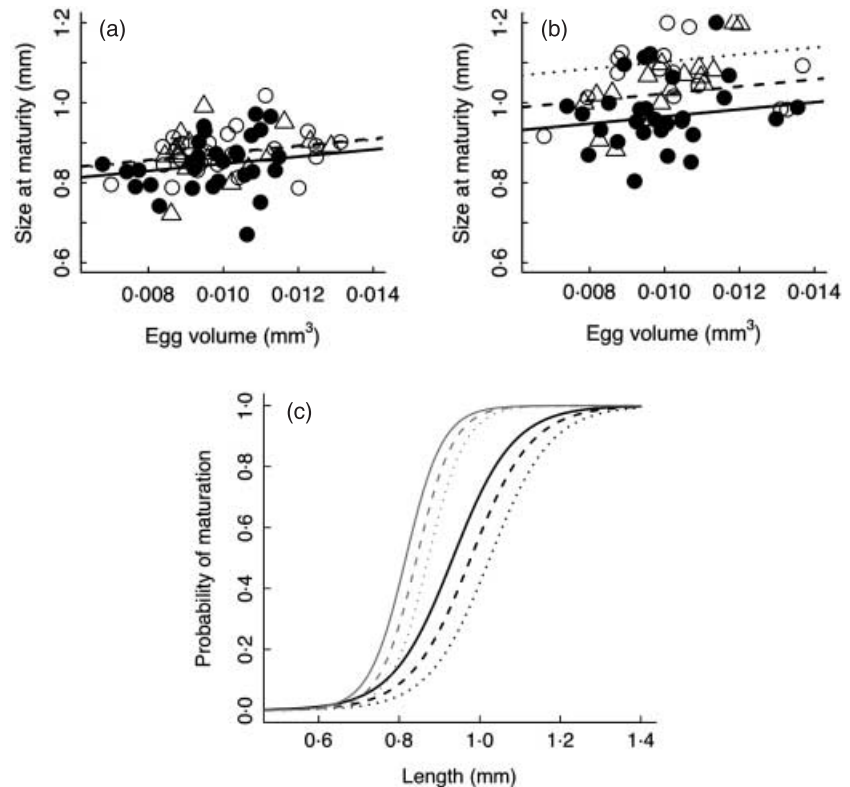
age had a bigger influence on the probability of maturing at a given size than egg volume (d.f. = 2, LRT = 12.91,  $P = 0.002$ , Fig. 4c).

#### MATERNAL-AGE EFFECTS: POPULATION LEVEL

Having shown that maternal age, in addition to egg size, influences individual life histories, we are now interested in whether such maternal effects can also influence transient population dynamics.

We collected eggs from mothers cultured under conditions designed to minimize age-related variation in egg size. The mean egg sizes from Day 3 or Day 9 mothers were not significantly different and represent typical egg sizes seen in the population (Day 3:  $0.1749 \pm \text{SD } 0.011$  mm, Day 9:  $0.1791 \pm 0.013$ ,  $F_{1,8} = 1.64$ ,  $P = 0.24$ ; Population mean egg size  $0.1801 \pm 0.015$ , estimated from  $n = 4052$  eggs measured over the course of the experiment, see Fig. 5a, inset).

As in the individual life-history study, the initial cohorts of eggs coming from 3-day-old mothers and 9-day-old mothers hatched at different rates. Eggs from Day 3 mothers typically hatched synchronously between days 3 and 4 after laying, with 6%, 7%, 79% and 6% hatching over days 1–4. Eggs from Day 9 mothers were more variable in their hatching, with 4%, 25%, 40% and 24% hatching each day from initiation (Fig. 5a). These difference in hatching rate, coupled with differences in development time, led to significant differences in the maturation time of the first cohort of adults (Fig. 5b) and subsequent differences in the timing of laying of the  $F_2$  generation eggs



**Fig. 4.** Size at maturity of males (a) and females (b) depends upon the size of egg that they hatched from, as well as the age of their mother when the egg was laid. 2-day-old mothers are shown as solid circles with a solid line; 5-day-old mothers are shown as clear triangles with a dashed line, and 8-day-old mothers are shown as clear circles with a dotted line. The lines represent the predictions from the best fitting linear mixed effects model (see text). (c) Consequently, the predicted probability of a juvenile maturing at any given size for males (grey lines) and females (black lines) decreases as mothers get older (2-day-old mothers, solid lines; 5-day-old mothers, dashed lines; 8-day-old mothers, dotted lines). See text for details of model selection.

(Fig. 5a), with the populations initiated with eggs of Day 3 females laying eggs much earlier than those initiated from eggs of Day 9 females.

The differences in the timing of the  $F_1$  cohort remains evident in the  $F_2$  cohort's maturation times, with the Day 3 treatments maturing significantly earlier than the Day 9 treatments (Figs 5c, 6). Marked generation cycles (created by relatively synchronous maturation of a whole generation) are strongly evident for the  $F_1$  and  $F_2$  generations for the populations initiated from both Day 3 and Day 9 mothers. However, after the 2nd generation cycle, the dynamics of the Day 3 populations settles to a (noisy) equilibrium (as shown by other longer-running experiments e.g. Benton & Beckerman 2005). In contrast, two further generation cycles (which partly overlap) are evident for the Day 9 populations. As a result, the most significant differences in the dynamics between the Day 3 and Day 9 populations occur during the  $F_3$  and  $F_4$  generations (Figs 5c and 6). The time series show the greatest differences around day 80 (Fig. 6) and are significantly different from around day 60 to day 90 when assessed by bootstrapping the difference between treatment means (Fig. 5c). The non-overlap of confidence intervals also indicates significant differences within this period (Fig. 5c).

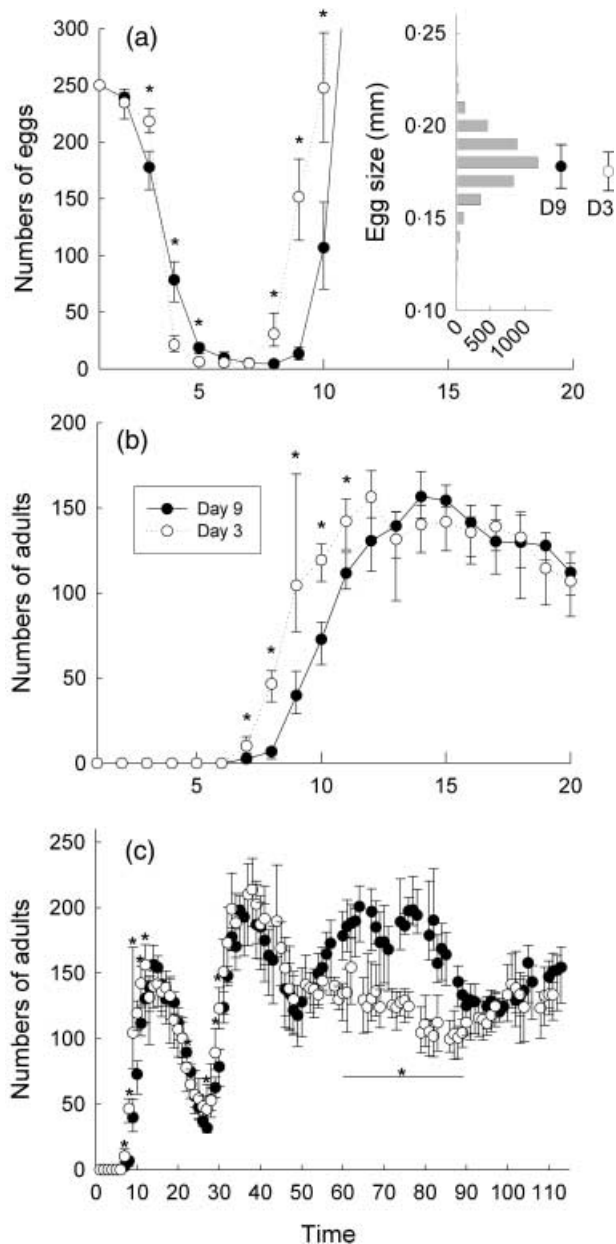
Are the population dynamical effects created by age-related maternal effects comparable in timing and magnitude to those created by egg size-related maternal effects? In a previous experiment, we charted the difference in transient population dynamics created from populations initiated from cohorts of 'large' vs. 'small' eggs (created by differences in food levels in mothers of the same age; data from Benton *et al.* 2005). In Fig. 6, we compare the magnitude of the maternal

effects for the 'egg-size effect' vs. the 'maternal-age effect' by calculating the daily difference between the average 'large' and 'small' egg dynamics and the 'old' and 'young' egg dynamics. On average, the magnitude of treatment differences is similar between the two experiments, with an average daily difference of 19% (between populations initiated with 'large' or 'small' eggs) vs. 22% (for populations initiated from Day 3 vs. Day 9 mothers) (Mann-Whitney,  $W = 5066$ ,  $P = 0.47$ ). While there are some differences in the dynamics between the experiments, notably in the time the maximum treatment difference occurs, the broad pattern is similar: the largest divergence between treatment groups occurs after 2+ generations have passed (two to three generations for the size-based effects, two to four generations in the current experiment). This must indicate that, although there are differences in the exact way that the initial maternal effects are propagated over time, the amplification of the initial differences occurs by broadly similar routes over broadly similar time-scales.

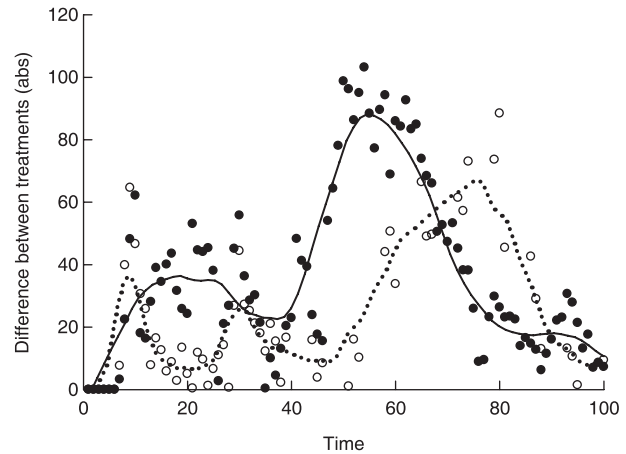
## Discussion

Population dynamics arise from the summed life histories of all the individuals in the population. To understand dynamics, especially in situations where there is environmental change, requires insights into the origins of variation between individuals. This variation may arise from individuals experiencing different environments. However, there are an increasing number of studies that show variation between individuals arises through parental effects, representing the influence of previous environments (see Mousseau & Fox





**Fig. 5.** Differences in the transient dynamics for populations initiated from 250 eggs from Day 3 mothers (red) and Day 9 mothers (black). (a) Egg counts showing initial differences in hatching rates of the  $F_1$  cohort and subsequent different laying dates for the  $F_2$  cohort. (b) Adult counts, showing how initial differences in hatching rates translates into differences in maturation of the  $F_1$  cohort. (c) Adult time series for the duration of the experiment. The initial differences in (a) and (b) propagate to create much larger differences between the  $F_3$  and  $F_4$  cohorts. Inset to (a) shows distribution of egg sizes from all measured eggs in population experiment (plus and minus SD). There is no significant difference in the size of eggs from Day 3 or Day 9 eggs, and both are representative of the central tendency of the distribution of egg sizes laid in free-running populations. Points represent the means of the five replicates in each treatment. Error bars are 95% bias-corrected and adjusted bootstrap confidence limits. \* represent significant differences between means (as inferred from bootstrap resampling), with  $_*$  indicating that adjacent points are different.



**Fig. 6.** The population dynamics' results from Fig. 5c are summarized as the (absolute) difference between the daily mean numbers of adults for Day 3 and Day 9 treatments (white dots). To illustrate the general pattern and describe the data, a nonparametric smoother (a 0.15 span loess) was fitted (dotted line). The black dots and solid line summarize a similar experiment (Benton *et al.* 2005) where the initial eggs were either 'large' or 'small' but from mothers of the same age (cf. eggs of the same size but from mothers of different ages). The maternal effects on dynamics are of similar magnitude.

1998 for a review). Variation in propagule size is an obvious, and now well-studied, reason for variation in offspring development, so, in this study we controlled for propagule size and investigated whether mothers could affect offspring performance in ways that are phenotypically less visible. We found that maternal age had a marked affect on hatching time, juvenile development and probability of maturation at a given size. As shown in the experiment using populations founded with cohorts of similarly sized eggs from mothers of different ages, these changes in offspring demographic rates affect population dynamics. We conclude that phenotypically indistinguishable variation in eggs – arising from age-related maternal effects – can lead to profound differences in population trajectories.

The route by which egg-size-independent maternal effects arise is unknown in this system. Although there is a known correlation between egg size and egg bulk protein (Benton *et al.* 2005), it is possible that there are subtle changes in the nutritional composition of eggs that are not detectable by size alone. Alternatively, there could be foetal programming unrelated to egg size, mediated by hormonal or enzymatic changes (Groothuis *et al.* 2005; Dloniak, French & Holekamp 2006; Martinez-Padilla 2006; Zhang *et al.* 2006), or a combination of the two. Earlier experiments explored maternal influences on offspring life histories in many different environments and indicated that egg size influences offspring life histories in different ways depending on the environment, as the juveniles may trade-off growth against survival or future fecundity differently depending on resources in the juvenile environment (Plaistow *et al.* 2006). That study showed, contrary to the expectation that maternal effects would be strongest under resource-limited environments, that maternal effects were always present but that different traits were

affected depending on maternal resources. That eggs of the same size, and from mothers of the same age, can lead to different offspring life histories in different environments suggests that the maternal effects arise, partially at least, as a result of differential use of resources in the eggs rather than differential provisioning of eggs.

Why do maternal-age effects exist? Other studies have shown that offspring fitness (especially longevity) decreases with maternal age (the 'Lansing effect'), often ascribed to senescence (Priest, Mackowiak, & Promislow 2002). In mites, the effects of maternal age on offspring life history, coupled with the associated age-related changes in egg size, lead to late-laid offspring that on average hatch later and mature at a larger size after a longer development. As explored in more detail elsewhere (Plaistow *et al.* 2007), this pattern of provisioning is consistent with an adaptive hypothesis that maximizes the fitness of offspring by adjusting their competitiveness (i.e. adult size) to the environment they mature into. The competitiveness of the offspring environment is related to the mother's reproductive output; late-laid eggs face a more competitive environment than early-laid eggs, and by maturing at a larger size the offspring are better competitors. Controlling for egg-size variation, maternal age is known to affect offspring life history in other invertebrates (McIntyre & Gooding 2000a,b; Giron & Casas 2003; Fox, Bush & Wallin 2003) and some vertebrates (Berkeley *et al.* 2004; Gorman & Williams 2005; Groothuis *et al.* 2005; Navara *et al.* 2006; Rubolini *et al.* 2006; Saino *et al.* 2006), although it is not clear whether these effects are an adaptive part of a lifetime offspring provisioning strategy, or are due to senescence (Priest *et al.* 2002).

The population experiment showed that the egg-size-independent maternal effects on life histories lead to marked differences in the transient population dynamics. This population dynamical effect is propagated over several generations by two interacting mechanisms. First, the initial differences in life history lead to different population structures which result in differences in the strength of density-dependent processes. This affects competition and per capita resources thereby creating resource-driven life-history variation. Second, the initial maternal effects influence the size and age at maturity of the  $F_1$  cohort, which itself creates variation in individuals' reproductive allocation (egg size, composition and number) leading to different maternal effects in the different populations. In support of this, controlling for random effects, tube, time and sample size, eggs in Day 9 populations were on average about 4% larger than those in Day 3 populations (0.1702 cf. 0.1638 mm) and they gave rise to females that were also about 4% larger (0.772 cf. 0.744 mm). The two mechanisms interact and reinforce each other as the differences in density-dependence have a greater effect if the individuals also come from eggs with different characteristics (see Plaistow *et al.* 2006). Additionally, any change in egg size leads to changes in fecundity as there is an egg size/egg number trade-off (Benton *et al.* 2005) which will directly affect the competitive environment into which the eggs hatch, leading to an indirect maternal effect (Beckerman *et al.* 2006). As a result of these reinforcing mechanisms, the largest numerical differences in

population sizes occur two to four generations after the initiation of the populations. The magnitude and pattern of the population dynamical effects resulting from initial age-based maternal effects are comparable to those resulting from initial size-based maternal effects (see Fig. 6, and Benton *et al.* 2005). This suggests that, in a population context, maternal effects (egg-size dependent or maternal-age dependent) are similar in magnitude, and that differences in initial conditions, whatever the cause, lead to differences in the dynamics that propagate far beyond the initial perturbation. Under constant conditions, our model system displays equilibrium dynamics after the initial transients decay away (see Benton & Beckerman 2005), hence this experimental design examines the population trajectories on the approach to equilibrium. Our expectation is that the population dynamics would converge on the same dynamical state. However, any population living under natural conditions would experience stochastic fluctuations in the environment which will frequently perturb the dynamics away from equilibrium conditions (and this will be especially true in the mites in the soil, where they are thought to exploit ephemeral food patches and exhibit 'boom and bust' dynamics). The population dynamics of many natural populations are therefore likely to be 'quasi-equilibrium' and be dominated by transients. In such cases, maternal effects, created by successive cohorts of adults that have developed under different conditions, may be instrumental in determining the near-term population trajectories even if they have little impact on asymptotic population behaviour.

Population dynamics has traditionally concentrated on numerical patterns, assuming that within the population (or stage/age class), individual variation mattered little. Here, we have shown that deterministic differences in eggs, which are not obviously related to their size and so may be undetectable in a population setting, may have a significant effect on population dynamics. Variation in reproductive allocation over a cohort's lifetime, can lead to deterministic changes in growth, survival and maturation of the next generation. This, in turn, changes the stage structure, the population size, and therefore the competitive environment. Changing access to resources further affects growth and survival leading to the propagation of effects across generations. This mechanistic causation underlying population variation is, in principal, both deterministic and understandable (see Plaistow *et al.* 2006). A modelling exercise concentrating on numerical fluctuations, without understanding the underlying life-history variation, would inevitably conclude that the dynamics had a strong random component.

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## References

- Beckerman, A.P., Benton, T.G., Lapsley, C.T. & Koesters, N. (2003) Talking 'bout my generation: environmental variability and cohort effects. *American Naturalist*, **162**, 754–767.



- Beckerman, A.P., Benton, T.G., Lapsley, C.T. & Koesters, N. (2006) How effective are maternal effects at having effects? *Proceedings of the Royal Society B: Biological Sciences*, **273**, 485–493.
- Benard, C., McCright, B., Zhang, Y., Felkai, S., Lakowski, B. & Hekimi, S. (2001) The C-elegans maternal-effect gene *clk-2* is essential for embryonic development, encodes protein homologous to yeast Tel2p and affects telomere length. *Development*, **128**, 4045–4055.
- Benton, T.G. & Beckerman, A.P. (2005) Population Dynamics in a Noisy World: Lessons from a Mite Experimental System. *Advances in Ecological Research*, **37**, 143–181.
- Benton, T.G., Plaistow, S.J., Beckerman, A.P., Lapsley, C.T. & Littlejohns, S. (2005) Changes in maternal investment in eggs can affect population dynamics. *Proceedings of the Royal Society B: Biological Sciences*, **272**, 1351–1356.
- Benton, T.G., Plaistow, S.J. & Coulson, T.N. (2006) Complex population dynamics and complex causation: devils, details and demography. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, **273**, 1173–1181.
- Berkeley, S.A., Chapman, C. & Sogard, S.M. (2004) Maternal age as determinant of larval growth and survival in marine fish, *Sebastes melanops*. *Ecology*, **85**, 1258–1264.
- Branicky, R., Benard, C. & Hekimi, S. (2000) *clk-1*, mitochondria, and physiological rates. *Bioessays*, **22**, 48–56.
- Coulson, T., Benton, T.G., Lundberg, P., Dall, S.R.X. & Kendall, B.E. (2006) Putting evolutionary biology back in the ecological theatre: demographic framework mapping genes to communities. *Evolutionary Ecology Research*, **8**, 1155–1171.
- Dloniak, S.M., French, J.A. & Holekamp, K.E. (2006) Rank-related maternal effects of androgens on behaviour in wild spotted hyaenas. *Nature*, **440**, 1190–1193.
- Drake, A.J., Walker, B.R. & Seckl, J.R. (2005) Intergenerational consequences of fetal programming by in utero exposure to glucocorticoids in rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, **288**, R34–R38.
- Forchhammer, M.C., Clutton-Brock, T.H., Lindstrom, J. & Albon, S.D. (2001) Climate and population density induce long-term cohort variation in northern ungulate. *Journal of Animal Ecology*, **70**, 721–729.
- Fox, C.W., Bush, M.L. & Wallin, W.G. (2003) Maternal age affects offspring lifespan of the seed beetle, *Callosobruchus maculatus*. *Functional Ecology*, **17**, 811–820.
- Gasparini, J., McCoy, K.D., Staszewski, V., Haussy, C. & Boulinier, T. (2006) Dynamics of anti-Borrelia antibodies in blacklegged kittiwake (*Rissa tridactyla*) chicks suggest maternal educational effect. *Canadian Journal of Zoology*, **84**, 623–627.
- Giron, D. & Casas, J. (2003) Mothers reduce egg provisioning with age. *Ecology Letters*, **6**, 273–277.
- Gorman, K.B. & Williams, T.D. (2005) Correlated evolution of maternally derived yolk testosterone and early developmental traits in passerine birds. *Biology Letters*, **1**, 461–464.
- Groothuis, T.G.G., Muller, W., Von Engelhardt, N., Carere, C. & Eising, C. (2005) Maternal hormones as tool to adjust offspring phenotype in avian species. *Neuroscience and Biobehavioral Reviews*, **29**, 329–352.
- Harrell, F.E. Jr. (2006) Hmisc: Harrell Miscellaneous. R package version 3.0-12. <http://biostat.mc.vanderbilt.edu/Hmisc>.
- Lindstrom, J. (1999) Early development and fitness in birds and mammals. *Trends in Ecology & Evolution*, **14**, 343–348.
- Martinez-Padilla, J. (2006) Prelying maternal condition modifies the association between egg mass and T cell-mediated immunity in kestrels. *Behavioral Ecology and Sociobiology*, **60**, 510–515.
- McIntyre, G.S. & Gooding, R.H. (2000b) Egg size, contents, and quality: maternal-age and -size effects on house fly eggs. *Canadian Journal of Zoology*, **78**, 1544–1551.
- McIntyre, G.S. & Gooding, R.H. (2000a) Effects of maternal age on larval competitiveness in house flies. *Heredity*, **85**, 480–489.
- Mousseau, T.A. & Fox, C.W. (1998) *Maternal Effects as Adaptations*. Oxford University Press, Oxford, UK.
- Navara, K.J., Siefferman, L.M., Hill, G.E. & Mendonca, M.T. (2006) Yolk androgens vary inversely to maternal androgens in eastern bluebirds: an experimental study. *Functional Ecology*, **20**, 449–456.
- Paitz, R.T., Harms, H.K., Bowden, R.M. & Janzen, F.J. (2007) Experience pays: offspring survival increases with female age. *Biology Letters*, **3**, 44–46.
- Pelletier, F., Clutton-Brock, T., Pemberton, J., Tuljapurkar, S. & Coulson, T. (2007) The evolutionary demography of ecological change: linking trait variation and population growth. *Science*, **315**, 1571–1574.
- Pianka, E.R. & Parker, W.S. (1975) Age-specific reproductive tactics. *American Naturalist*, **109**, 453–464.
- Plaistow, S.J., Lapsley, C.T. & Benton, T.G. (2006) Context-dependent intergenerational effects: the interaction between past and present environments and its effect on population dynamics. *American Naturalist*, **167**, 206–215.
- Plaistow, S.J., St Clair, J.J.H., Grant, J.D. & Benton, T.G. (2007) How to put all your eggs in one basket: empirical patterns of offspring provisioning throughout mother's lifetime. *American Naturalist*, **170**, 520–529.
- Priest, N.K., Mackowiak, B. & Promislow, D.E.L. (2002) The role of parental age effects on the evolution of aging. *Evolution*, **56**, 927–935.
- R Development Core Team (2005). *R: Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. Available from URL <http://www.R-project.org>.
- Räsänen, K. & Kruuk, L.E.B. (2007) Maternal effects and evolution at ecological time scales. *Functional Ecology*, **21**, 408–421.
- Rubolini, D., Romano, M., Martinelli, R. & Saino, N. (2006) Effects of elevated yolk testosterone levels on survival, growth and immunity of male and female yellow-legged gull chicks. *Behavioral Ecology and Sociobiology*, **59**, 344–352.
- Saino, N., Ferrari, R.P., Romano, M., Martinelli, R., Lacroix, A., Gil, D. & Moller, A.P. (2006) Maternal allocation of androgens and antagonistic effects of yolk androgens on sons and daughters. *Behavioral Ecology*, **17**, 172–181.
- Zehnder, C.B., Parris, M.A. & Hunter, M.D. (2007) Effects of maternal age and environment on offspring vital rates in the oleander aphid (Hemiptera: Aphididae). *Environmental Entomology*, **36**, 910–917.
- Zhang, T.Y., Bagot, R., Parent, C., Nesbitt, C., Bredy, T.W., Caldji, C., Fish, E., Anisman, H., Szyf, M. & Meaney, M.J. (2006) Maternal programming of defensive responses through sustained effects on gene expression. *Biological Psychology*, **73**, 72–89.

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