Transgenerational effects of food availability on age at maturity and reproductive output in an asexual collembolan species

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Transgenerational effects of environmental conditions can have several important ecological and evolutionary implications. We conducted a fully factorial experiment manipulating food availability across three generations in the collembolan Folsomia candida, a springtail species that inhabits soil and leaf litter environments which vary in resource availability. Maternal and grandmaternal food availability influenced age at maturity and reproductive output. These effects appear to be cumulative rather than adaptive transgenerational life-history adjustments. Such cumulative effects can profoundly influence eco-evolutionary dynamics in both stable and fluctuating environments.

Keywords: maternal effect; adaptive plasticity; resource variability

1. INTRODUCTION
In both animals and plants, the environment of the mother often has an influence on the developmental trajectory of her offspring that is additional to the influence of their own environment [1]. Although reports remain quite rare, environmental effects can also carry across several generations. For example, two consecutive days of exposure to a 1 h cold shock in adult Drosophila reduces developmental time in both offspring and grand-offspring [2]. In heterogeneous environments, this transgenerational plasticity and multi-generational ‘phenotypic memory’ may be maintained by selection if an ancestor’s experience of the environment can help its descendants to refine their phenotypes in anticipation of the environmental conditions that they are likely to experience [3,4]. Surprisingly few studies have performed the factorial experiments that are necessary to track offspring performance across different combinations of grandmaternal, maternal and offspring environments. This approach is necessary to properly assess the potential for adaptive maternal and grandmaternal effects and, perhaps more significantly, it can also reveal positive or negative cumulative effects of past environments that may contribute to population age structure and growth and even to the probability of local extinction (e.g. [5–7]).

Using a fully factorial design, we show that resource availability not only influences life-history traits within generations in the collembolan Folsomia candida, but that it also influences fundamental life-history traits of ecological and evolutionary significance for at least three generations.

2. MATERIAL AND METHODS
(a) Study organism and rearing conditions
Folsomia candida (Collembola: Isotomidae) is a cosmopolitan species that is widely used in studies of ecology and toxicology [8]. To eliminate variation caused by genetic differences, a single strain of F. candida that is entirely homozygous and effectively clonal was used in all the experiments. All collembolans of this strain were parthenogenetically descended from a single mother collected in 2004 at Buttes-Chaumont Park (Paris, France). To evaluate the extent to which the results found for this strain were generalizable, a further three strains were studied using the protocols described below but at reduced replicate sizes of four to seven individuals per treatment and these results are presented in the electronic supplementary material. Collembolans were housed individually in sealed vials that provided a circular substrate that was 380 mm^3 in size and made from a 4:1 mixture of plaster of Paris and powdered charcoal. The substrate was moist such that the humidity in the vials was 100 per cent and temperature was maintained at 21 ± 0.5°C.

(b) Experimental procedure
Two feeding regimes were used systematically to vary the food available. In the high food regime, the pelletized food (made from a 10:1 mixture of yeast and agar) was available ad libitum. In the low food treatment, food was available only for the first 24 h of each 4 day period. A fully factorial design was used to apply all combinations of the two feeding regimes to three consecutive collembolan generations. In the first or parental generation (P), each food regime (high food, H and low food, L) was applied for 46 days before obtaining eggs from the treated collembolans to found the first filial generation (G1). In the second or filial generation (G2), eight treatments were employed to account for the regimes previously experienced by both the P and G1 generations: HHH, HHL, LHH, LHL, LH, HL, H, L, G. The eggs from the first clutches of G1 mothers were used to produce the second filial generation (G2). In G2, eight treatments were employed to account for the regimes previously experienced by both the P and G1 generations: HHH, HHL, LHH, LHL, LH, HL, H, L, G. Each generation was replicated 30 times in the G1 (n = 120 individuals) and 25 times in the G2 (n = 200).

Egg sizes (i.e. the average diameter of the spherical eggs at laying) and body sizes (i.e. the distance from the vertex of the head to the tip of the abdomen, measured on the day of hatching and every 4 days thereafter until day 46) were obtained through measurements of high-resolution photographs. Collembolans were monitored daily. Our focal life-history traits are: (i) clutch size, (ii) reproductive output over 46 days, (iii) length at hatching, (iv) length at maturity (v) time to maturity, and (vi) time to death (if appropriate).

Data were tested for homoscedasticity and normality of error terms and Box-Cox transformed when necessary before being analysed with full general linear models. Effect sizes for pairwise contrasts are given by the standardized Cohen's f statistic calculated from the bias-reducing df metric [9,10]. Effect sizes for pairwise contrasts are given by the standardized Cohen's d statistic with 95% confidence intervals. Post hoc comparisons were made with the Ryan-Einot-Gabriel-Welsch procedure. Binomial data were analysed by logistic regression and mortality data were analysed by Cox regression.

3. RESULTS
High food availability reduced the age at maturity (figure 1(a)). In the G_3 time to maturity was...
significantly influenced by an interaction between the parental and the current environments ($F_{1,99} = 5.89$, $p = 0.02$, Cohen’s $f = 0.14$). In the G2, time to maturity was affected by an interaction between the P and G1 environments ($F_{1,184} = 4.17$, $p = 0.04$, Cohen’s $f = 0.11$) as well as the separate effect of the current environment ($F_{1,184} = 98.07$, $p < 0.001$, Cohen’s $f = 0.70$). Collembolans on high food matured equally quickly regardless of the environments of their ancestors but time to maturity for those on low food was influenced by the environments of the two preceding generations (figure 1c). Post hoc tests demonstrated that collembolans on low food which had both mothers and grandmothers that were on high food (i.e. the HHL treatment) took longer to mature than collembolans on low food, which had mothers that were on high food but grandmothers that were on low food (i.e. the LHL treatment).

Collembolans on low food were approximately 20 per cent shorter in length at maturity than those on high food (MD = 302.78 ± 32.97 μm, Cohen’s $d = 2.60$, 95% CI = 2.22–2.99, $F_{1,184} = 320.91$, $p < 0.001$, Cohen’s $f = 1.29$). The transgenerational factors that influenced time to maturity were not found to produce accompanying differences in size at maturity (influence of maternal environment: $F_{1,184} = 2.30$, $p = 0.13$, Cohen’s $f = 0.05$; influence of grandmaternal environment: $F_{1,184} = 0.36$, $p = 0.55$, Cohen’s $f < 0.01$; figure 1b,d). Furthermore, neither time to maturity nor size at maturity was significantly influenced by egg size, hatchling length, mother’s or grandmother’s size at maturity, the size of the mother’s or the grandmother’s first clutch or the total number of eggs produced by the mother or grandmother ($p > 0.15$ in all cases).

In the G2, both the maternal environment ($F_{1,184} = 5.12$, $p = 0.02$, Cohen’s $f = 0.09$) and the current environment ($F_{1,184} = 375.27$, $p < 0.001$, Cohen’s $f = 1.36$) influenced the number of eggs in the first clutch (figure 2a). In addition, the interactions between the P and G2 environments ($F_{1,184} = 3.50$, $p = 0.062$, Cohen’s $f = 0.07$) and the G1 and G2 environments ($F_{1,184} = 3.30$, $p = 0.070$, Cohen’s $f = 0.06$) were marginally non-significant. Collembolans on high food tended to lay more eggs in their first clutch if their mothers were also recipients of high food. Collembolans on high food which had both mothers and grandmothers on low food (i.e. in the LLH treatment) laid an average of 4.33 ± 2.64 fewer eggs than those in the LHH treatment.

**Figure 1.** (a,b) Time to maturity and size at maturity in the G1 and (c,d) in the G2. Mean values and 95% confidence intervals are indicated. In (a,b), treatment codes on the x-axes indicate the sequence of food treatments over two consecutive generations: P and G1. In (c,d), treatment codes on the x-axes indicate the sequence of food treatments over three consecutive generations: P, G1 and G2. The groups of treatment means identified by post hoc comparisons are indicated by uppercase letters.
(Cohen’s $d = 1.11$, 95% CI = 0.51 – 1.71) and 3.53 ± 2.72 fewer eggs than those in the HHH treatment (Cohen’s $d = 0.85$, 95% CI = 0.25 – 1.44). The number of eggs laid in the first clutch was lower for collembo-lans on low food and no effect of maternal or grandmaternal environment was detected in the four low-food treatments that were applied in the G2 (figure 2a). Furthermore, collembo-lans on high food regimes also tended to produce more clutches (MD of 0.86 ± 0.26 clutches, $t_{190} = 6.54$, $p < 0.001$, Cohen’s $d = 0.94$ with 95% CI of 0.64 – 1.24) and there was evidence that this effect spanned the generations (figure 2b) as the main effects of the P environment ($F_{1,184} = 4.44$, $p = 0.036$, Cohen’s $f = 0.12$), G1 environment ($F_{1,184} = 6.17$, $p = 0.014$, Cohen’s $f = 0.15$) and G2 environment ($F_{1,184} = 43.78$, $p < 0.001$, Cohen’s $f = 0.46$) were all significant (while $p > 0.35$ for all interactive terms). However, this effect on number of clutches did not translate into differences in reproductive success (as measured by eggs produced) as the G2 environment was the only significant effect ($F_{1,184} = 489$, $p < 0.001$, Cohen’s $f = 1.60$; $p > 0.24$ for all other terms in the model). Finally, high food availability increased mortality within a generation (by up to 9.4 times, 95% CI = 2.14 – 41.51, Wald$_d = 19.01$, $p < 0.01$) but had no detectable influence across generations (maternal influence: Wald$_d = 0.92$, $p = 0.34$; grandmaternal influence: Wald$_d = 0.46$, $p = 0.50$).

Quantitatively and qualitatively similar results were recorded for the other three strains (and these results are set out in the electronic supplementary material).

4. DISCUSSION
Our data provide evidence for maternal and, more notably, grandmaternal effects on life-history trajectories in collembo-lans. Given that this species (i) is likely to encounter variable food conditions in the wild [8], (ii) is potentially prone to strong responses to environmental variation [11], and (iii) is free of parent–offspring conflict because its reproduction is effectively clonal, it is tempting to speculate that the effects may be adaptive across generations. However, the patterns of age at maturity and reproductive output do not fit simple adaptive scenarios. Indeed, with the caveat that our data do not fully capture life-time reproductive success, there is no evidence for the adaptive transgenerational plasticity that would be predicted if food availability fluctuated on timescales similar to generation times. Two results are particularly important. First, our factorial design did not suggest increased reproductive output or survival for matched versus mismatched environments across two generations. Second, G2 offspring from lineages with consistent low food availability over the previous generations were not fitter when raised on low food compared with G2 offspring with a high-food ancestry. Instead, with the exception of age to maturity, the maternal effects on reproductive output suggest a cumulative negative effect of poor maternal nutrition on the number of reproductive events (but with no detectable corresponding effect on total reproductive output). Given a transgenerational run of high-quality environments, the evolutionarily favoured strategy may be to produce more frequent clutches that are smaller in size. In comparing age at maturity of the HHL and LHL treatments, we would also tentatively suggest that, for individuals directly experiencing poor environments, having a parent or grandparent that experienced a similarly poor environment may convey the advantage of early reproductive maturity.

Interestingly, none of the maternal and grandmaternal effects was mediated via egg size or any other likely morphological trait or life-history trait. This suggests that changes in offspring developmental trajectories are mediated more subtly (e.g. via epigenetic regulation or changes in egg composition). Such effects may be mediated partly via maternal age. Because collembo-lans in the low food treatment were older at the time for reproduction, age per se may influence life-history trajectories of offspring. Indeed, maternal age effects on offspring age at maturation, reproductive output and senescence have been documented in

Figure 2. Mean number ± 95% confidence intervals of (a) eggs laid in the first clutch and (b) clutches laid during the first 46 days of life by collembo-lans within the various treatments of the G2. Treatment codes on the x-axes indicate the sequence of food treatments over three consecutive generations: P, G1 and G2. The groups of treatment means identified by post hoc comparisons are indicated by uppercase letters.
several species (e.g. [12,13]) and may be cumulative. Our experiment does not allow us to statistically disentangle these effects because age and treatment are strongly linked. However, we predict that environments in which food availability is low will, at minimum, select for maintenance of any age effect because they tend to increase age at maturity while reducing early reproductive output.

Our study is novel for comprehensively demonstrating how food availability influences life histories across all possible combinations of environments over three generations. This demonstration has uncovered previously unappreciated transgenerational effects including a particularly pervasive effect on age at maturity. Our findings also provide timely empirical validation of the caveat based on theoretical considerations that, while it may be tempting to view maternal effects in terms of the adaptive benefit they might convey to offspring, the greatest benefit will most often be derived directly by the parent itself [14]. We also note that maternal and grandmaternal effects are subtle relative to the much larger environmental effects that an individual experiences directly during its lifetime and that it is not entirely clear how results obtained for asexual organisms will generalize for sexual organisms. That said, transgenerational life-history effects like those that we have quantified have potentially profound implications for population dynamics, as has been demonstrated for soil mites [6,7,15]. Arthropod study systems may often be ideal for assessing individual plasticity within the contexts of environmental variation, population dynamics and evolution.