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Parental ecological history can differentially modulate parental age effects on offspring physiological traits in *Drosophila*

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Abstract

Parents adjust their reproductive investment over their lifespan based on their condition, age, and social environment, creating the potential for inter-generational effects to differentially affect offspring physiology. To date, however, little is known about how social environments experienced by parents throughout development and adulthood influence the effect of parental age on the expression of life-history traits in the offspring. Here, I collected data on *Drosophila melanogaster* offspring traits (i.e., body weight, water content, and lipid reserves) from populations where either mothers, fathers both, or neither parents experienced different social environments during development (larval crowding) and adulthood. Parental treatment modulated parental age effects on offspring lipid reserves but did not influence parental age effects on offspring water content. Importantly, parents in social environments where all individuals were raised in uncrowded larval densities produced daughters and sons lighter than parental treatments which produced the heaviest offspring. The peak in offspring body weight was delayed relative to the peak in parental reproductive success, but more strongly so for daughters from parental treatments where some or all males in the parental social environments were raised in crowded larval densities (irrespective of their social context), suggesting a potential father-to-daughter effect. Overall, the findings of this study reveal that parental ecological history (here, developmental and adult social environments) can modulate the effects of parental age at reproduction on the expression of offspring traits.

Key words: indirect fitness, life-history, maternal effects, paternal effects.

Inter-generational effects are processes through which parents pass on non-genetic information of their environment to their offspring, with long-lasting fitness effects to both generations (Mousseau and Dingle 1991; Mousseau and Fox 1998; O'Dea et al. 2016). The exchange of information from parents to offspring can increase or decrease offspring (and consequently, parents') fitness if the offspring environment matches (or mismatches) the parental environment, or if non-genetic effects transferred by the parents improve (or hampers) the ability of the offspring to cope with its environment (Monaghan 2008; Engqvist and Reinhold 2016; Champagne 2020). This can modulate population dynamics and influence eco-evolutionary processes acting in local populations (Qvarnström and Price 2001; Benton et al. 2008). Either way, inter-generational effects modulate the expression of offspring traits based on parental signals (Engqvist and Reinhold 2016). Inter-generational effects are widespread in nature and have been described in plants (Agrawal et al. 1999), invertebrates (Fox et al. 1997; Valtonen et al. 2012; McNamara et al. 2014; Wilson and Graham 2015; Qazi et al. 2017; Morimoto et al. 2017a, 2017b), and vertebrates such as fish (Schade et al. 2014; Stratmann and Taborsky 2014), lizards (Uller et al. 2005; Rozen-Rechels et al. 2018), birds (Bouwhuis et al. 2010, but see Pei et al. 2020), and mammals including humans, for example, Hasselquist and Nilsson

(2009), Dantzer et al. (2013), see also Uller et al. (2013) for a meta-analysis.

Parental age is known to affect offspring lifespan and more generally, performance, and fitness, whereby older parents produce offspring with overall shorter lifespan and overall lower quality or fitness, which is broadly known as "Lansing effect" (Lansing 1947; Monaghan et al. 2020, but see also Comfort 1953). To date, there has been a range of complex results reported in the literature, showing that overall (grand-) mothers' and fathers' age at reproduction modulate (grand-) offspring fitness across 1 or multiple generations. For instance, in insects, older mothers produce offspring with shorter lifespan (Lansing effect *sensu stricto*) but the effects of mothers' age on offspring fitness traits such as developmental time, mass at maturity, and fecundity are less consistent, with some taxa showing either an increase or decrease in trait expression, or no maternal effects (see e.g., Table 3 in Zehnder et al. 2007, for summary). Even within species, inter-generational and trans-generational effects are known to differ depending on ecological factors. In the oleander aphid Aphis nerii, the maternal age at which offspring mass at maturity was maximized depended on host plant species, with mothers fed *Asclepias syriaca* producing heavier offspring on Day 6 in comparison to Day 11 when mothers were fed *Asclepias viridis* (Zehnder et al. 2007).

Inter-generational and trans-generational effects interact with biotic and abiotic ecological factors to shape offspring life-history (Gibbs et al. 2010; Ducatez et al. 2012; Wylde et al. 2019). For instance, in the butterfly *Pararge aegeria*, larval mass declined with maternal age but this decline was less strong when females were forced to fly (as an experimental manipulation to mimic dispersal) (Gibbs et al. 2010). Likewise, in the butterfly *Pieris brassicae*, offspring from fathers that were forced to fly and mated with old mothers showed longer developmental times than control fathers with old mothers, this effect increased with paternal age, but paternal effects (both in terms of flight and age) on offspring development were absent when mothers were young (Ducatez et al. 2012). Interestingly, the same study found that paternal effects were more accentuated in the offspring at the larval stage while for mothers, the effects were exacerbated in the adult stage of the offspring (Ducatez et al. 2012), potentially suggesting a de-coupling of parental effects across life-stages in holometabolous insects. In the neriid fly *Telostylinus angusticollis*, grand-offspring lifespan decreased with grandparents' reproductive age in a similar fashion for both grandmother and grandfather lines, and this effect was independent of dietary effects in an intervening generation (Wylde et al. 2019). Overall, these studies highlight the complexity of inter- and trans-generational effects but also those ecological factors experienced by the parental generation can either mitigate or accentuate these effects in future generations.

Ecological factors experienced by the parents can influence inter- and trans-generational effects of age by directly or indirectly altering parental reproductive investment. Evidence suggesting condition-dependent parental reproductive investment and/or inter-generational effects continue to grow. In *Drosophila melanogaster* parents can modulate their reproductive investment, timing, and overall reproductive success (i.e., offspring number) in response to the presence and number of (male) rivals (Bretman et al. 2012), male and female age (Qazi et al. 2017; Morimoto et al. 2017a, 2017b; Sepil et al. 2020), male and female developmental conditions (i.e., diet, conspecific density) (Valtonen et al. 2012; Wigby et al. 2015; Morimoto et al. 2016, 2017a, 2017b), as well as partners' size, age, and mating status (Pitnick 1991; Lüpold et al. 2010; Turiegano et al. 2013). Furthermore, inter- and trans-generational effects in *D. melanogaster* have been described in terms of ancestral diet composition and quality (Dew-Budd et al.

2016; Deas et al. 2019; Emborski and Mikheyev 2019) as well as conspecific larval density (Valtonen et al. 2012; Morimoto et al. 2017a, 2017b). Inter- and trans-generational effects on offspring life-history traits have also been described in other insect groups, including grasshoppers (Franzke and Reinhold 2013), wasps (Morag et al. 2011), flies other than *D. melanogaster* (Crean et al. 2014; Wylde et al. 2019), butterflies (e.g., Ducatez et al. 2012; see also review by Woestmann and Saastamoinen 2016) and beetles (Lock 2012; Macagno et al. 2018), attesting to the ubiquity of inter- and trans-generational effects in insects (Zehnder et al. 2007). To date, however, we still do not know whether parental developmental and adult social environments—both of which are known to modulate evolutionary forces such as sexual selection (Morimoto et al. 2016)—can affect the expression of fitness-related traits in the offspring, nor whether these effects are constant or differentially affected by parental age at reproduction.

In this study, I collected new data on offspring traits from previously published work, where I had assembled artificial populations of *D. melanogaster* at equal sex ratios in which fathers, mothers, none, or both parents were reared in high and low larval density and experienced varying social environments ("parental treatments") (Morimoto 2017a; Figure 1a). This newly collected offspring data allowed me to gain insight into the following question: Do parental developmental and adult social environments modulate the effect of parental age on offspring traits? More specifically, the data allowed for the study as to whether the peak in parental reproductive success, which was originally measured in Morimoto et al. (2017a, 2017b), coincides with the time where offspring trait (related to fitness) expression was also maximum. This allowed me to test whether (1) parental offspring number coincides with offspring quality (a parents' reproductive "golden age") where both the number and size of offspring are maximized or (2) there is a trade-off between offspring number and size above and beyond parental developmental and adult social environments (Akhund-Zade et al. 2021).

Materials and Methods

The original purpose of this experimental design was to address how developmental and social effects can influence population traits (Morimoto et al. 2017a, 2017b). However, offspring of these experiments were stored and could be
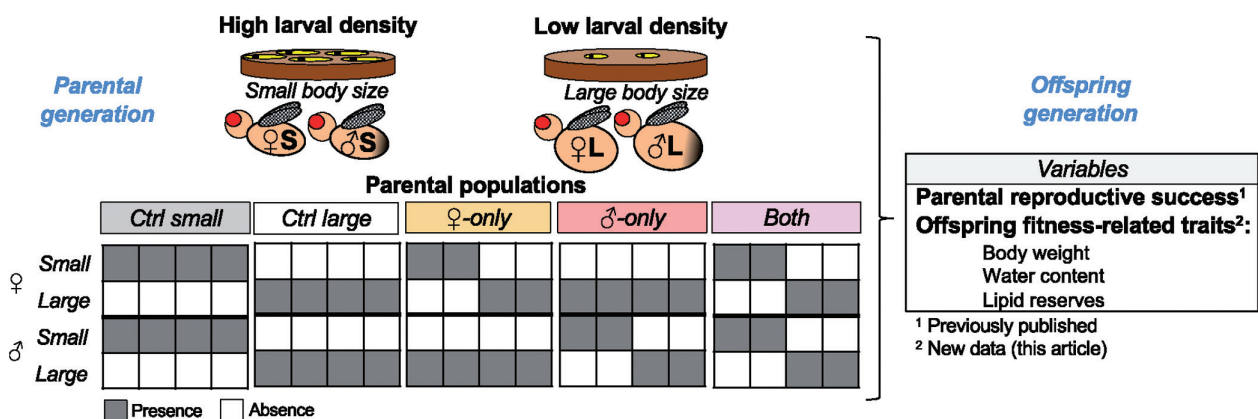
Figure 1. Schematic representation of the experimental design. I varied parental larval density to create small and large flies, which were then assembled in groups of 8 (4 males and 4 females) with varying social compositions. Design originally conceptualized to investigate parental reproductive success in Morimoto et al. (2017a).

retrieved for analyses of body composition, which allowed me to gain insights into how parental developmental and adult social environments modulate offspring trait expression. Below, I provide a brief description of the experimental design, for which the details can be found at length in a previous publication (Morimoto 2017a, 2017b).

Fly stock and parental developmental and adult social environments manipulations

Wild-type inbred *OregonR* stock of *D. melanogaster* was maintained in large populations (> 5,000 individuals) in cages with overlapping generations for >10 generations. All fly stocks were maintained and all experiments conducted at 25°C on a 14:10 light:dark cycle in a controlled humidified room (humidity = 68%) and fed with standard sugar–yeast–maize–molasses medium with excess live yeast granules. I manipulated parental developmental environment by means of relative changes in parental body size based on larval crowding: the crowded individuals (small body size adults) were from vials with ~ 50 larvae/mL of food (~ 200 larvae/34 mL vial containing ~ 4 mL fly food) whereas the uncrowded individuals (large body size) were from vials with ~4 larvae/mL of food (~ 40 larvae/34 mL vial containing ~10 mL fly food). Parental groups with mixed social compositions were assembled with 4 males (fathers) and 4 females (mothers) (i.e., 8 individuals per group), which were randomly selected from a pool of >1,000 individuals of each sex and mixed into 5 parental treatments (N = 17 replicates per parental treatment) as following (Figure 1):

1. Control small. Adult social group where both mothers (*n = 4*) and fathers (*n = 4*) had small body size (i.e., from a crowded developmental environment);
2. Control large. Groups where both mothers (*n = 4*) and fathers (*n = 4*) had large body size (i.e., from an uncrowded developmental environment);
3. Female-only. Groups where all fathers were large (*n = 4*). Half of the mothers were large (*n = 2*) and the other half small (*n = 2*).
4. Male-only. Groups where mothers were large (*n = 4*). Half of the fathers were large (*n = 2*) and the other half small (*n = 2*).
5. Both sexes. Groups where half of the individuals were large and the other half, small for both sexes.

Note that this is not a full factorial design, and therefore the results have some limitations in terms of identifying the mechanisms underpinning the phenomena observed below. Nevertheless, both full and non-full factorial designs provide insights into the presence and to some extent, the magnitude of phenomena. This limitation is acknowledged in the "Discussion" section but does not invalidate the effects found in the study. These parental treatments were chosen for several reasons: (1) there is information in the literature about population-level responses in terms of harassment, fitness, and survival in these groups (see Morimoto et al. 2017a, which is the original experimental design for the data collected here). (2) I have previously shown that the strength of sexual selection is modulated by group composition in similar group treatments (Morimoto et al. 2016) and (3) There have been a substantial number of studies in the literature investigating how crowding and/or social environment influence life-history and reproductive traits in *D. melanogaster*

(e.g., Amitin and Pitnick 2007; Bretman et al. 2009; Shenoi et al. 2016; Wigby et al. 2016; Bath et al. 2018; Hopkins et al. 2019; Dore et al. 2020; see also references in Morimoto and Pietras (2020)), which are useful for interpreting the results.

Parental groups were allowed to interact freely. Groups were transferred to fresh vials with 6 mL of food on Days 3, 6, 9, 13, 16, 19, 23, 27, 35, 40, 45, and 50 after the onset of the experiment, and the old vials were reserved for 13–15 days until adult offspring emerged fully. Offspring were 3–5 days old. Females stopped producing offspring at approximately Day 35; see Morimoto et al. (2017a, 2017b). Offspring had food ad libitum and larval densities were always <20 larvae/g of diet which can be considered high density given the natural history of *D. melanogaster* (Morimoto and Pietras 2020). I nevertheless included a proxy of offspring crowding—that is, total parental reproductive success per time point—as a fixed effect in the analyses (see details below). For every time point, we scored the number of surviving females and males in all populations. This procedure was repeated until all mothers of the groups died, a point where the group was considered extinct. I then assessed parental group reproductive success by counting the total number of adult offspring in each parental treatment per time point (Supplementary Figure S1).

Body weight and composition

I measured offspring body weight, water content, and lipid composition under the assumption that offspring with high body weight, water content, and lipid reserves translate into higher fitness (Fairbanks and Burch 1970; Honěk 1993; Than et al. 2020). In flies, physiological traits such as body weight, water content, and lipid reserves are correlated with desiccation and starvation resistance, as well as male and female reproductive success (Fairbanks and Burch 1970; Partridge et al. 1987; Da Lage et al. 1989; Honěk 1993; van Herrewege and David 1997; Gibbs and Markow 2001; Nestel and Nemny-Lavy 2008; Stefana et al. 2017; Morimoto et al. 2019; Than et al. 2020), and thus can be useful proxies to assess how parental inter-generational effects can affect offspring fitness. Adult offspring were separated into 2 cohorts. In the first cohort, 6–9 randomly selected sons and 6–9 randomly selected daughters *per* replicate parental group (i.e., N = 17) *per* parental treatment *per* time point were measured for wet body weight using a Sartorius® ME5 scale (0.0001 g precision) (N_{total} = 1458). In the second cohort from the same treatments, 5 sons and 5 daughters *per* treatment *per* time point until Day 19 (for logistic reasons) were randomly selected from a subset of 6 replicate populations *per* treatment (also randomly selected), dried in the oven for 48 h at 60°C to eliminate water content and weighed as described above (dry weight). Dried flies were individually allocated to 10 mL glass tubes where we performed lipid extraction with chloroform (Sigma Aldrich®, St. Louis, MO, USA, Cat no. 288306) as described in Morimoto et al. (2019). Flies were again dried for 48 h at 60°C and weighed as described. Percentage of lipid for individual flies was estimated as the difference between dry weight and the weight after lipid extraction divided by the dry weight × 100 (N_{total} = 270). Water content was measured by subtracting the average offspring body weight *per* vial *per* parental treatment *per* day (N_{total} = 78).

Statistical analysis

All statistical analyses were performed in R software version 3.6.2 (Team RDC 2010). I used linear mixed models
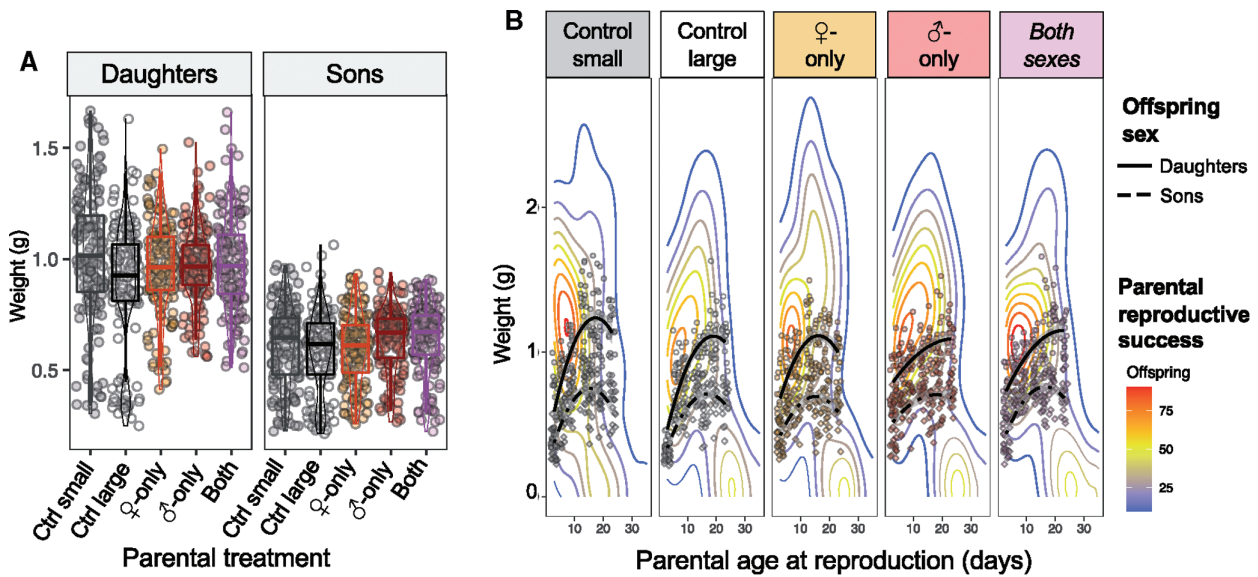
Figure 2. Parental reproduction and offspring traits. (A) Parental treatment effects on adult daughters' and sons' body weight (in grams) adapted from Morimoto et al. (2017a). (B) Offspring body weight (in grams) in relation to parental age at reproduction (in days) and parental treatment. Contour lines correspond to the pattern of parental reproductive success, whereby red contour regions represent peak parental reproductive success (second y-axis with parental reproductive success was omitted for clarity but raw data is presented in Supplementary Figure S1). Trend lines plotted using the "lm" function in R. Circles: Daughters; Diamond: Sons.

from the 'lme4 v.1.1-23' and 'lmerTest v.3.1-2' packages for all the analyses (Bates et al. 2007; Kuznetsova et al. 2017). Population vial was fitted as a random effect in all models, whereas the 3-way interactions between parental treatment, offspring sex, and the linear and quadratic (non-linear) effects of parental age at reproduction were included as fixed effects; P-values were obtained from F-statistics using the inbuilt 'ANOVA' function (type III). I also included parental total reproductive success *per* vial *per* time interval, which was extracted from previously published work (Morimoto et al. 2017a, 2017b), as a fixed effect in all models. This metric was used as a proxy of offspring "crowding" which allowed me to control for any potential confounding effects of offspring intraspecific competition on offspring traits (see e.g., review; Than et al. 2020). This approach assumed a somewhat linear relationship between crowding and trait expression which, for the purpose of a controlling variable, this is not unreasonable (see e.g., Horváth and Kalinka 2016, where linear terms could fairly well describe non-linear effects which occur at densities > 20 eggs per mL of diet). Moreover, offspring larval densities were > 20 larvae/g of diet, which could be considered high density given the natural history of *D. melanogaster* (Morimoto and Pietras 2020), and thus, unlikely to have reached sufficient high densities to potentially trigger major non-linear effects. To obtain the estimated peak (in days) of parental reproductive success and offspring weight along parental age, I calculated the point in which the second derivative of the general linear models fitted to the data was equal to zero, for each sex separately. Confidence intervals (CIs) were calculated using bootstrapping with 1,000 iterations in the 'boot v.1.3-25' package (Canty 2002). Because bootstrapping assumes a normal distribution of errors, in some cases the lower CI limits were negative. In these instances, negative CI values were rounded to zero days. All plots were made using the 'ggplot2 v.3.3-1' package (Wickham 2016).

Results

Parental developmental and adult social environments differentially affect offspring body weight

Offspring crowding had a significant negative effect on offspring body weight ($F_{1,1045.5} = 4.052$, $P = 0.044$) but not on offspring water content ($F_{1,47} = 0.787$, $P = 0.380$) or lipid reserves ($F_{1,232} = 1.559$, $P = 0.213$). After controlling for these effects, daughters were heavier than sons (Sex: $F_{1,1402.1} = 51.422$, $P < 0.001$, Figure 2A), but not necessarily with higher water content (Sex: $F_{1,47} = 0.510$, $P = 0.479$) or lipid reserves (Sex: $F_{1,234.4} = 0.069$, $P = 0.793$). The linear an5d non-linear relationships between offspring body weight and parental age at reproduction were differentially affected by parental treatment (Linear * Treatment: $F_{1,1390} = 18.624$, $P < 0.001$; Non-linear * Treatment: $F_{1,1380.7} = 13.382$, $P < 0.001$, Supplementary Table S1), whereby there was a steeper linear and more accentuated curvilinear relationship between parental age and body weights in Control Small, Control Large and Female-only relative to Male-only and Both sexes parental treatments (Figure 2B). Linear (but not non-linear) effects of parental age influenced offspring weight (Linear * Sex: $F_{1,1402} = 11.251$, $P < 0.001$), whereby the effects of parental age on the linear increase in offspring weight was more pronounced in daughters than sons (Figure 2B). In fact, the Control Large parental treatment (where mothers and fathers were large) produced daughters (mean ± *SD*: 0.903 ± 0.254) and sons (mean ± *SD*: 0.594 ± 0.160) that were ca. 12% and 10% lighter, respectively, compared with the parental treatments that produced the heaviest offspring of each sex (namely, Control Small for daughters, 1.015 ± 0.283, and Both sexes for sons, mean ± *SD*: 0.657 ± 0.128, see also Supplementary Text S1). I also found a 3-way interaction between parental age at reproduction, parental treatment, and offspring sex on offspring weight. This emerged because the differential effect of parental

treatment on the relationship between parental age at reproduction and offspring weight was more pronounced in daughters than in sons (Linear * Treatment * Sex: $F_{1,1402} = 3.266$, $P = 0.011$; Non-linear * Treatment * Sex: $F_{1,1402} = 2.409$, $P = 0.048$) (Figure 2B).

Whereas there were neither effects of sex, nor the interactions between sex, parental treatment, and the linear and non-linear effects of parental age on offspring water content and lipid reserves (Supplementary Table S1), there were main linear and non-linear effects of parental age at reproduction on offspring water content (Linear: $F_{1,47} = 11.245$, $P = 0.002$; Non-linear: $F_{1,47} = 9.804$, $P = 0.003$) and lipid reserve (Linear: $F_{1,235} = 6.935$, $P = 0.009$; Non-linear: $F_{1,235.1} = 9.228$, $P = 0.003$), suggesting that the linear and non-linear effects of parental age on offspring physiological traits were similar for sons and daughters of all parental treatments (i.e., neither statistically significant 2- nor 3-way interactions).

Peak in parental reproductive success does not necessarily coincide with peak offspring body weight

The overall and sex-specific peak estimates with their CI are shown in Table 1 (reproductive success data reproduced from Morimoto et al. 2017a, 2017b). In general, offspring body weight reached peak expression later than parental reproductive success for all treatments. The average magnitude of the delay in peak offspring weight relative to parental peak in reproductive success was more evident for daughters in the Control Small, Male-only, and Both sexes treatment (although with relatively large CIs for the latter 2 treatments). This suggests that when at least some males in social conditions have experienced poor developmental environments, there is a delay in daughters' peak in body weight as the reproductive age of the parent increases (Table 1, Figure 2B, and Supplementary Figure S2).

Discussion

Here, I collected new data from a previous experiment which allowed me to gain insights into the following question: does parental developmental and adult social environment modulate the effect of parental age on offspring traits? I found that all parental treatments resulted in delays a daughters' peak in body weight relative to the parental peak in reproductive success, but that this delay is particularly more accentuated for treatments where the social contexts of fathers contained all (4) or some (2) individuals that experienced a crowded (poor) developmental condition (i.e., Control Small, Male-only, and Both sexes) (Table 1). Paternal effects (either in daughters' or sons', or both) have been previously described across taxa (including humans) in the literature (Pembrey et al. 2006; Whitelaw 2006; Nelson et al. 2010; Hughes 2014). Inter-generational effects on offspring, especially daughters' body weight such as those found in this study (Table 1 and Figure 2a and b) can generate long-term fitness consequences to the parents (via indirect fitness) and the offspring (via direct and indirect fitness). This is because in *Drosophila*, as in the majority of insects, body weight and size are positively correlated with fitness (Honěk 1993; Than et al. 2020). Thus, over the course of the offspring's reproductive lifetime, the small differences in body weight originating from inter-generational effects found here have the potential to accumulate and result in large net differences in direct mating and reproductive success (i.e., fitness) of the offspring (and indirect fitness to the parents) (e.g., Partridge and Farquhar 1983; Partridge et al. 1987; Honěk 1993; Chapman and Partridge 1996; Bangham et al. 2002; Galipaud et al. 2013; Wigby et al. 2015; Morimoto et al. 2016). Further studies should test whether small body size differences carried over from 1 generation to the next are indeed translated into differences in fitness, or whether body size differences are counterbalanced by other behavioral processes (e.g., increased male harm toward larger and more attractive females; Long et al. 2009).

Table 1. Estimates of peak parental reproductive success and offspring body weight

Trait	Sex	Parental treatment	Peak estimate (day)	lwr 95% CI	upr 95% CI	Delay diff.
Parental reproductive success	—	Control small∞	0	0	9.36	—
	—	Control large	13.7	11.1	15.8	—
	—	Female-only	11.4	8.09	13.2	—
	—	Male-only	11.4	8.26	13.2	—
	—	Both sexes	10.3	6.07	13.3	—
Offspring body weight	Daughters	Control small	21.9	18.7	26.8	21.9
	—	Control large	18.4	17.5	20.7	4.7
	—	Female-only	16.2	15.5	17.3	4.8
	—	Male-only	24.3	16.7	73.8	12.9
	—	Both sexes	24.1	19.7	40.4	13.8
	Sons	Control small	17.8	16.1	20.2	17.8
	—	Control large	16.2	15.4	18.4	2.5
	—	Female-only	15	14.1	16.5	3.6
	—	Male-only	18.5	0	35.8	7.1
	—	Both sexes	16	15.2	17.5	5.7

Notes: For consistency, I fitted a quadratic (non-linear) term for all models even though in some cases the relationship between parental age and reproductive success or offspring traits was linear (highlighted with the symbol ∞, see Supplementary Figure S1). CIs were calculated using bootstrapping (1,000 replicates). Delay difference was calculated by subtracting the estimated peak of offspring weight from the estimated peak in parental reproductive success.

The data show that offspring trait expression varied over the parental reproductive lifespan (Figure 2B), which supports the idea that some offspring may have higher "fitness value" to the parents than others (Smith and Fretwell 1974; Haig 1990) see also Wolf and Wade 2001). This provides supporting evidence for the broader concept of the Lansing hypothesis as defined in Monaghan et al. (2020) which states that parents age modulates offspring quality and fitness. I found that parental reproductive age affected all of the offspring physiological traits including body weight (Figure 2), water content, and lipid reserves (Supplemental Figure S2). Higher lipid reserves and water content are known to increase survival under stress in flies (Fairbanks and Burch 1970; Djawdan et al. 1998; Reim et al. 2006; Ballard et al. 2008; Klepsatel et al. 2016). Thus, in stressful environments, offspring with higher expression of these traits have higher direct fitness due to better odds of surviving and reproducing and also have higher indirect fitness value to their parents (see above). The fact that offspring trait expression varied over the parental reproductive lifespan in this study suggests that there may exist a trade-off between parental investment in offspring traits and the expression of other (parental or offspring) traits, otherwise all offspring should for example be as heavy as possible under the correlation of body size with fitness (Honek 1993; Than et al. 2020). The molecular mechanisms underpinning the temporal variation in inter-generational effects remain to be explored, but it is in theory possible that maternal effects via mRNAs are transferred to the egg/embryo at different quantities and/or translated at different rates after fertilization. Evidence in mice has revealed that temporal patterns play a key role in maternal mRNA effects (Alizadeh et al. 2005) and in *Drosophila*, the level of histone gene expression is known to be at least partly modulated by the quantity of maternal mRNA (Anderson and Lengyel 1980) (see also broader recent reviews in the topic by Pálfy et al. 2017 and Vastenhouw et al. 2019). This highlights the potential temporal dynamism underpinning inter-generational effects which requires further investigations.

The decline in parental reproduction with age ("reproductive senescence") is a widespread phenomenon in nature (Ivimey-Cook and Moorad 2020), although species display different patterns of reproductive senescence throughout lifespan (Jones et al. 2014). A recent theoretical model suggests that reproductive senescence in mothers' fecundity can be under different selective pressures than maternal effects, leading to a potential dissociation of senescence effects in these traits (Moorad and Nussey 2016). From my understanding, 1 implication of this model pertinent to the findings presented here is that the decline in offspring production across replicate populations should not necessarily coincide with (senescence in) inter-generational effects on offspring traits. In this study, the data do not allow for direct inferences on senescence of inter-generational effects (unless assuming that offspring traits are entirely modulated by inter-generational effects) but it nevertheless shows that parental reproductive senescence effects are to some extent dissociated from the expression of offspring traits. These results appear to indirectly support the predictions of the model, with the caveat that in this experimental design I could not differentiate maternal (for which the model was explicitly developed) or paternal effects, or the interaction between both.

I found that parental developmental and social conditions modulate the effects of parental age of reproduction on offspring traits (Figure 2 and Supplementary Figure S2). Thus, independent of the underpinning molecular mechanisms, the data presented here provide suggestive evidence of a putative condition-dependent Lansing effect on offspring fitness-related traits, whereby parental condition (e.g., amount of resource acquired during development) modulates the effects of parental age on offspring trait expression. A previous study in another fly showed that diet effects in an intervening generation in a multi-generational had no contribution to the grand-parental age effects in the grand-offspring (Wylde et al. 2019). However, multi-generational studies in *Drosophila* showed that sugar and fat dietary manipulations—as well as diet quality—in ancestral parental diet modulated sex-specific physiological and reproductive traits in the offspring and grand-offspring (Dew-Budd et al. 2016; Deas et al. 2019; Emborski and Mikheyev 2019). In this study, we manipulated crowding experienced by the parental generation, and crowding is known to reduce nutrient availability (Klepsatel et al. 2018) but also generates changes in diet and individual microbiome (Henry et al. 2020) as well as nutrient composition of the diet (Nguyen et al. 2019). Therefore, it is possible that crowding experienced during the parental generation triggers physiological responses (only partly related to diet) which in turn, modulate the effects of parental age at reproduction on offspring trait expression. More studies are needed, both in terms of molecular mechanisms and inter-generational effects, to uncover how crowding affects individual physiology in the present and future generations.

This study investigated how phenotypic variability in adult parental populations, emerging from different larval crowding regimes, modulate parental effects on offspring fitness traits. Natural populations of *Drosophila* species display substantial variation in adult body size (Pétavy et al. 2001; Gibert et al. 2004; Morimoto and Pietras 2020), which likely modulates the opportunities for inter-generational effects above and beyond variations in environmental conditions. Thus, our findings provide insights into parental effects on offspring traits in an ecologically relevant design. Phenotypic variability is widespread in nature and underpins physiological effects and social interactions that determine the evolutionary trajectory of populations (Willmore et al. 2007; Chevin et al. 2010; Geiler-Samerotte et al. 2013; Maynard et al. 2019). Moreover, phenotypic variability in the parental population can be transferred to the offspring (Bonduriansky and Crean 2018), thereby influencing the adaptability of the offspring to environmental conditions whereas also resulting in joint correlation between offspring and parental traits (Wolf and Brodie 1998; Stanton et al. 2000). Thus, the findings presented here can guide future studies on the inter-generational effects of parental developmental and adult social environments in other (non-model) species.

It is worth mentioning that the findings presented here need to be interpreted with caution, because the study has the limitation of not being full factorial and using indirect proxies of offspring fitness. Nevertheless, this study corroborates previous studies which highlight the complexity of generational effects in insects (Ducatez et al. 2012; Wylde et al. 2019), and contributes to the field by adding a new perspective as to how parental developmental and adult social environments modulate parental age effects on offspring trait expression. It is also worth mentioning that alternative explanations and criticisms for the findings have been proposed, amongst which the most pertinent is (1) the inability to assign whether mothers of each

offspring were large or small, which precludes me from knowing whether all females contributed to the offspring pool (e.g., only large females laid eggs in mixed size social treatments) and (2) the lack of precise control on the larval density of the offspring, opening up the possibility that more fecund females had lighter offspring due to larval crowding (not inter-generational effects). Detailed responses to these points are given in Supplementary Text S1 in the supplementary information but in summary: (1) it is extremely unlikely that 1 class of females (e.g., small) would not reproduce in the presence of the other class (e.g., large), given the biology of *Drosophila* females as well as the data observed here and in previous studies where large and small female reproduction was measured after exposure to rivals (Supplementary Text S1 and Supplementary Figure S3, Morimoto et al. 2016) and (2) the predictions for offspring weight assuming that larval crowding and/or the trade-off offspring number-size was driving the effects are inconsistent with the observed data for offspring weight (Supplementary Text S1 and Supplementary Figure S4). Therefore, it is likely that the effects presented here, albeit limited in experimental design, constitute an important advance in our understanding of how parental ecological history can influence inter-generational effects.

In conclusion, this study shows that parental ecological history—in this study, parental developmental and adult social environments—can differentially modulate the effects of parental age at reproduction on the expression of offspring traits. The data show that the peak in parental reproductive success does not necessarily coincide with the peak offspring trait, suggesting that offspring from the same parents produced at different times can contribute to parents' fitness differently.

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Conflict of Interests Statement

The author has no conflict of interest to declare.

Data Accessibility Statement

Raw data are available in Supplementary Table S2. R script with code is provided as supplementary material.

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

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