Age-related shifts in dietary and oviposition preferences and steep costs of modest parental ageing in *Drosophila melanogaster*

Oonagh Barker*[1], Edward R. Ivimey-Cook[1], Wilfried Haerty[2], Alexei A. Maklakov[1] and Tracey Chapman[1]

1 School of Biological Sciences, University of East Anglia, Norwich, Norfolk, UK, NR47TJ

2 Earlham Institute, Norwich Research Park, Norwich, Norfolk, UK, NR4 7UZ

*Corresponding author: o.barker@uea.ac.uk

Abstract

Nutritional requirements vary across sexes and throughout an organism's lifetime, and are predicted to influence dietary preferences to maximise fitness. However, the consequences of age-related changes in dietary preferences within and across generations are unknown. This is an important omission, as age-related changes in nutrient intake could have direct, sex-specific effects on offspring. We addressed this using the fruitfly *Drosophila melanogaster* to measure age-specific choice among three protein:carbohydrate (P:C) diets in both sexes. Simultaneously, we measured age-specific oviposition site choices and tracked offspring development, survival and dietary preferences. Older females showed stronger preferences for protein than younger females, and older males spent less time on balanced and high protein diets than younger males. Females preferred to oviposit on balanced P:C, however preference for high carbohydrate increased with age. Third instar larvae of older parents preferred balanced P:C, while larvae of younger parents showed no overall preference. Unexpectedly, offspring of 15-day-old parents showed slower development and higher preadult mortality than those of 5-day-old parents, revealing novel effects of only a modest increase in parental age. These findings highlight interdependence between reproductive ageing and nutrient selection, and suggest an underappreciated interaction between parental age and early nutrition in shaping offspring life history.

Introduction

The evolution of sex- and age-specific dietary preferences confers the opportunity for individuals to selectively forage for optimal combinations of nutrients to maximise their reproductive potential (Raubenheimer et al., 2022; Simpson et al., 2004; Simpson & Raubenheimer, 2012). In insects, males and females often maximise their reproductive output at different intakes of carbohydrate (C) relative to protein (P), leading to contrasting dietary preferences which can underpin sex-specific variation in lifespan (Adler et al., 2013; Jensen et al., 2015; Lee et al., 2008; Maklakov et al., 2008; Reddiex et al., 2013). Sex- and life-stage specific dietary responses have been comprehensively described in the fruit fly *Drosophila melanogaster*, which has long been recognised as a valuable model for biogerontology and for the study of the interplay between diet and ageing in particular (Bruce et al., 2013; Kapahi et al., 2017; Piper & Partridge, 2018; Regan et al., 2016).

In male *D. melanogaster*, both longevity and reproduction are maximised on high C diets (Jensen et al., 2015; Lee et al., 2008). In contrast, in females, while lifespan is also maximised on high C, reproductive output is maximised on diets containing high P (Bruce et al., 2013; Camus et al., 2017; Jensen et al., 2015; Le Couteur et al., 2016; Lee, 2015; Lee et al., 2008). There are also sex-specific effects in dietary responses to mating. Unmated individuals of both sexes prefer to feed along a P:C ratio of 1:4 (Jensen et al., 2015). However, female, but not male, dietary intake changes significantly after mating (Barnes et al., 2008; Camus et al., 2018; Carvalho et al., 2006; Ribeiro & Dickson, 2010; Sydney et al., 2024; Yapici et al., 2008), but see Liu et al., 2024. Following mating, females ingest significantly more protein (Camus et al., 2018; Carvalho et al., 2006; Ribeiro & Dickson, 2010); (Barnes et al., 2008; Ribeiro & Dickson, 2010; Yapici et al., 2008). This dietary switch occurs as part of a suite of post-mating changes, and is mediated by a specific component of the male ejaculate – the 'sex peptide' (Barnes et al., 2008; Ribeiro & Dickson, 2010; Yapici et al., 2008).

The evolution of variable dietary preferences allows individuals to potentially select the optimal combination of nutrients according to age and lifestage. For example, in females, lifetime reproductive output could potentially be maximised by adaptively adjusting P and C intake to balance longevity and egg productivity according to each age. As high C maximises both lifespan and reproduction in males (Jensen et al., 2015; Lee et al., 2008), dietary preference for high C / low P is expected to be sustained throughout a male's lifetime. Senescence, or the process of physiological deterioration associated with increased probability of death and decreased reproductive performance with age, may impair the ability of organisms to adaptively optimise diet selection by affecting the pathways through which nutrients are

sensed and metabolised (dos Santos & Cochemé, 2024). Dietary optimisation throughout life may also be constrained because older flies of both sexes eat less and are less able to increase food intake to compensate for low dietary protein, with females more adversely affected in this regard than males (Rushby et al., 2023). This process is known as 'protein leveraging' and has been explored as a potential driver of obesity in humans (Raubenheimer & Simpson, 2023). Ultimately, sex differences in nutrient sensing over an individual's lifetime is likely to interplay with sex-specific reproductive senescence (Lemaître & Gaillard, 2017; Maklakov et al., 2009).

Diet choice affects not only the lifespan and reproductive output of the focal generation, but in species such as fruitflies that selectively lay eggs on food substrates, it also affects key fitness-related traits in offspring. Oviposition site selection is even considered a rudimentary form of parental care (Lihoreau et al., 2016a; Miller et al., 2011). Female *D. melanogaster* are reported to assess the concentration and chemical identity of sweet compounds (Otarola-Jimenez et al., 2025), show sensitivity to mechanosensory properties (Yapici, 2020; Zhang et al., 2020), and respond to the microbial environment (Fowler et al., 2025) when selecting oviposition sites. Oviposition site preference has been reported variously as favouring low P:C ratio oviposition sites (Lihoreau et al., 2016b; Rodrigues et al., 2015), higher P:C oviposition sites (Gray et al., 2018a), or that ovipositing females avoid sucrose (Chen et al., 2022; Otarola-Jimenez et al., 2025; Yang et al., 2008). The reason for this conflict is not clear, but females might be expected to prefer diets which speed up larval development and increase survival (Gray et al., 2018; Lihoreau et al., 2016). In addition, it is not known how female oviposition preferences might vary with age. It is possible that females could become more selective with age because egg quality potentially declines, which could place increased importance on compensatory feeding by larvae. Alternatively, older females could show reduced sensitivity to nutritional differences in oviposition sites due to age-related senescent declines in nutrient sensing. As developmental diet is an important determinant of early-life adult fecundity (Ruchitha et al., 2024), changes in oviposition site selection are likely to affect offspring quality. Beyond this direct effect of oviposition site selection on offspring fitness, the developmental diet of parents can have inter- and trans-generational effects on offspring (Deas et al., 2019; Emborski & Mikheyev, 2019; Triggs & Knell, 2012; Valtonen et al., 2012; Zizzari et al., 2016).

To date, the best-known example of inter-generational effects of parental age is known as the 'Lansing Effect', in which advanced maternal age is associated with reduced offspring lifespan. Evidence for Lansing Effects across animal taxa is patchy, with the most consistent effects reported in insects and rotifers (Ivimey-Cook et al., 2023). However, much less is known about how parental age effects are filtered through environments experienced by offspring. That such effects are potentially important is suggested by findings in the red flour beetle *Tribolium castaneum*, in which offspring of older parents are less resilient to starvation than offspring of younger parents, despite pupating later at a higher body mass

(Halle et al., 2015). Investigating how offspring of different parental ages are affected by rearing conditions, such as the nutritional environment, is therefore a potentially overlooked source of life history trait variation.

In the present study, we addressed the question of how dietary and oviposition preferences change with age and measured the resulting impact on offspring development and preadult survival. We used solid, semi-defined diets (Piper et al., 2014) in diet patch preference assays rather than capillary-based feeding assays (Ja et al., 2007) to enable cross-comparison of choices versus responses between life-stages. We measured the diet preferences of mated males and females at three different ages (5, 15, and 25 days old, which corresponded to early, mid, mid-to-late across the reproductive lifespan for females in this species). Over an interval of 4h, individuals were offered a free choice between 3 different P:C diet ratios (1:4, 1:1, and 4:1). In addition, we recorded oviposition site preferences of females by counting the number of eggs laid on each diet patch. Dietary preferences of larvae emerging from eggs laid by females of all 3 ages were then tested. Fitness effects of the different diets on offspring were tested by placing larvae of 5 and 15d (day old) mothers on one of each of the three test diets.

Our hypotheses were that there would be sex-, age- and lifestage-specific differences in diet preferences, and that diet choices exerted by mothers would have the potential to influence offspring fitness. We addressed these by testing the following predictions:

1. That males would consistently prefer the 1:4 P:C diet, which maximises both reproduction and lifespan (Jensen et al., 2015; Lee et al., 2008, p. 200) (Figure S1a).
2. That females would favour the 1:1 patch and possibly also visit the 4:1 patch for protein supplementation in early life to support high fecundity (Jang & Lee, 2018; Jensen et al., 2015), but that this preference would become less pronounced with age, as egg-laying decreases (Figure S1b).
3. That females would either lay eggs predominantly on the 1:1 patch which benefits larval development (Gray et al., 2018b; Lihoreau et al., 2016b; Rodrigues et al., 2015) or prefer the 1:4 patch in line with previous findings (Lihoreau et al., 2016b; Rodrigues et al., 2015) but that in either case the preference shown by 5d females would decline in 15 and 25d females due to reproductive senescence (Hamilton, 1966; Kirkwood & Holliday, 1979; Meyer et al., 2025; Rose, 1994).
4. That larvae would consistently favour the 1:1 patch, as this diet is reported to minimise their development time (Rodrigues et al., 2015) and improve survival and learning performance (Lihoreau et al., 2016b). Larvae were predicted to avoid the 4:1 patch as this was thought likely to represent a toxic excess of protein (Mirzaei et al., 2014; Tatar et al., 2014) .

5. That larvae reared directly on 1:1 P:C (predicted preferred) diets would show the highest survival, largest body sizes and fastest development, with the slowest development and smallest body sizes for larvae reared on 1:4, and the lowest survivorship on 4:1 as this likely represents a toxic excess of protein under long-term no-choice conditions (Mirzaei et al., 2014; Tatar et al., 2014).

Methods

Fly stocks

Experimental flies were sourced from a large, outbred population of wildtype Dahomey *Drosophila melanogaster* (Chapman et al., 1994). Stocks were maintained on a standard sugar-yeast-agar (SYA) diet (50g sugar, 100g brewer's yeast, 15g agar, 30ml 10% Nipagin, 3ml propionic acid, 970ml water) which represents a P:C ratio of approximately 1:2. Experimental flies were maintained throughout in a controlled environment with a 12:12h light:dark cycle at 25°C and 50% humidity. Eggs were collected from population cages on purple grape juice agar plates spread with a small quantity of live yeast paste. Egg collection plates were quartered and placed in 70ml bottles of SYA to rear the first generation. At 4 days (d) post eclosion, the first generation were transferred to egg collection cages with access to plates of yeasted purple grape juice agar and allowed to lay overnight. First-generation flies were discarded the following day and egg collection plates incubated for a further 24h, after which first instar larvae were picked into 7ml SYA vials at a density of 25 larvae per vial. This ensured that the second generation (used in choice assays) had standardised parental age, and that their offspring (used in larval choice assays and no-choice assays of preadult survival) had standardised grandparental age.

At 2d post eclosion, second-generation individuals were transferred to new vials containing 7ml SYA media under light CO_2 sedation. At this time, flies were sorted into mixed-sex groups of 10 males and 10 females per vial to eliminate any effect of uneven sex ratio. High survivorship was observed under these conditions, hence sex ratios were maintained effectively for the entire 25d period, with a maximum of 1-2 deaths observed per vial. Experimental adults from this cohort (N = 1250) were randomly sampled at 5, 15 and 25d post eclosion and divided between choice assays or egg collection cages (Figure S2). Media changes were carried out every 3-4d with the use of light CO_2 sedation, and occurred 2d before each choice assay to standardise time since exposure to CO_2 across all treatments. Experiments were carried out across a single block.

Experimental diets

Meridic diets (SI 4.1) were made following a protocol adapted from Piper et al. (2014) in which P and C are provided in the form of casein (from bovine milk) and sucrose, respectively. Diets were made with P:C ratios of 1:4, 1:1 and 4:1 with a combined macronutrient content (total grams of casein and sucrose) for each diet of 120g/L^{-1}.

Dietary choice assays

In all treatment groups, choice assays were conducted by allowing individuals a free choice between 3 2cm square cut-outs of 1:4, 1:1 or 4:1 media. All assays were conducted within 10cm diameter plastic petri dishes (Figure S3).

Dietary choice in mated males and females

Adult males and females (N = 900 each) were randomly sampled from the experimental population (N = 2000) at 5, 15, and 25d post eclosion and transferred to 15 choice assay plates per sex and age group, in single-sex groups of 10 under light CO_2 sedation (N total replicates = 90; Figure S3a). Following 1h of acclimatisation, plates were observed for 8 consecutive intervals of 30min, and the number of individuals gathered on each patch (or not on any patch) were counted at each interval.

Dietary choice in larvae

Eggs were collected from the adult cohort at 5, 15, and 25d post eclosion. First instar larvae (N = 450) were collected from plates of yeasted purple grape juice agar in groups of 10 and deposited at the intersection of three contiguous patches of media in the centre of a petri dish (Figure S3b). Plates were then covered and incubated for 48h, at which time larvae were manually extracted from media patches and counted (N = 15 replicate plates per parental age group, total N = 45). Larval dietary choice was determined by the number of larvae found in each diet choice patch after the 48h foraging period.

Oviposition choice

Following the adult dietary choice assays, plates of 10 females (N = 15 plates/ age group, total N = 45) were collected and incubated for 24h. After this, plates were frozen at -20°C for at least 4h to arrest egg development. Plates were then defrosted in batches for counting. The sides of each food patch were carefully cut away to a depth of approximately 1mm and laid flat so that each surface to which females had access and could lay eggs could be viewed from above in a single image. Photographs of patches containing eggs (N = 135) were taken using an Eagle-M microscope with a GXCAm screen. Egg counts were then conducted by eye using a counting tool in ImageJ v1.54i.

Offspring development - under ‘no choice’ conditions

Eggs were collected from the adult cohort at 5 and 15d post eclosion from the same egg collection plates as those used to source individuals for larval dietary choice assays. For each parental age group, five first instar larvae were transferred into 30 vials of 1:4, 1:1 and 4:1 P:C media (total N = 900). Plugs of media were 1cm deep and placed on top of 7ml agar containing 10ml Nipagin and 3ml Propionic acid per litre.

This ensured that the plugs of meridic media were sufficiently moist for the duration of larval development. Vials were monitored once every 2d until the appearance of pupae, after which vials were monitored daily for eclosed adults until day 14. This cut-off point was chosen as eclosion ordinarily peaks in the stock population after 9-10d and so would allow ample time for all viable individuals to eclose. Vials were then frozen at -8°C for subsequent measures of wing and thorax sizes (SI 2, Figure S4).

Statistical analyses

Statistical analyses were carried out in R v4.3.2 (The R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org). A table of model type by generation and trait (Table S6) and full model outputs (Table S7-15) are available in the SI. For all analyses, model fits, zero inflation and dispersion were checked using DHARMa v0.5.0 (Hartig & Hartig, 2017) and pairwise comparisons with Tukey correction performed using the R package emmeans v2.0.3 (Lenth, 2023). Observation-level random effects were used to control overdispersion when their inclusion improved model fit (Harrison, 2014).

Dietary choice assays in adults and larvae, oviposition choice assays, and differences in wing and thorax lengths among offspring reared under no-choice were analysed using the package glmmTMB v1.1.14 (Brooks et al., 2017). Diet choice in adult males and females, measured in the form of repeated counts of individuals over 8 time intervals, was analysed using a negative binomial generalised linear mixed effects model (GLMM). In larvae, where diet choice was measured at a single timepoint with limited zero inflation, better model fit was achieved using a Poisson GLMM. For oviposition choice, we constructed a two-column response matrix of 'successes' (an egg laid on a patch) versus failures (eggs laid on other patches) for each replicate. This approach allowed us to fit a beta-binomial distribution to account for the high zero inflation and overdispersion present in the dataset, which was largely attributable to the expected lower number of eggs laid by older versus younger females. Wing and thorax lengths (SI 1.2-1.3) were analysed using Gaussian LMMs, also in glmmTMB v1.1.14 (Brooks et al., 2017).

Development time, measured as the time in days taken by individual offspring to emerge as adults, was first analysed using a mixed effects Cox Proportional Hazards model which was fitted and checked using the R package Survival v3.8-6 (Therneau & Lumley, 2015). Due to non-adherence to proportional hazards, this was followed by a two-part event history analysis which modelled time-to-event (development time) and probability-of-event (the probability that offspring emerged as adults at all). Development time was modelled with a Conway-Maxwell Poisson distribution, which provided the best model fit given under dispersion, in glmmTMB v1.1.14 (Brooks et al., 2017). The 4:1 reared group was excluded from the analysis of development time (SI 1.1), as there were very few eclosions from 15d parents on this diet (N = 15 of a possible 150) which caused issues with model convergence. Probability of eclosion, which

included the 4:1 reared group, was analysed using a binomial GLMM using lme4 v2.0-1 (Bates et al., 2015).

Results

Sex- and age-specific variation in diet choice

Diet patch selection by adult males and females was determined by a three-way interaction between sex, diet, and age (X^2 = 100.6 , df = 6, p = <0.0001; Table S7).

Pairwise comparisons between age groups (Table S16) showed a striking age and sex difference in how males and females divided time on diet patches versus no patch at all. 25d males were less frequently observed on 1:1 (*25d – 5d* estimate = -0.95, p <0.0001; *15d - 25d* estimate = 1.29, p <0.0001) and 4:1 (*25d – 5d* estimate =-0.38, p <0.01; *15d – 25d* estimate = 0.40, p <0.01) diet patches, preferring no patch overall (*25d – 5d* estimate = 0.46, p <0.0001; *15d – 25d* estimate = 0.64, p <0.0001). However, interest in the 1:4 diet patch did not change with age in males, consistent with prediction #1 (*25d – 5d* estimate = -0.21, p = 0.29; *15d – 5d* estimate 0.04, p = 0.29; *15d – 25d* estimate = 0.25, p = 0.16).

Female preference for diet type varied significantly with age, while overall time spent on food patches versus no patch remained constant (*25d – 5d* estimate = 0.06, p = 0.39; *15d – 5d* estimate = -0.02, p = 0.88; *15d – 25d* estimate = -0.09, p = 0.17). The 1:4 diet patch was chosen more frequently by 15d females than by 25d females (*15d – 25d* estimate = 0.60, p <0.001). The 1:1 food patch was favoured more by 15d and 25d females than by 5d females (*25d – 5d* estimate = -0.67, p <0.0001; *15d – 5d* estimate = -0.44, p <0.001; *15d – 25d* estimate = 0.23, p = 0.18). Similarly, the 4:1 food patch was favoured equally by 15 and 25d females (*15d - 25d* estimate = -0.01, p = 0.99), but less so by 5d females (*25d – 5d* estimate = 0.40, p = 0.001; *15d - 5d* estimate = 0.39, p = 0.002). These findings contradict prediction #2, as females did not reduce occupancy of the P-rich diet patches as they aged, but instead older females increased visits to P-rich patches relative to the youngest group (Figure 1).

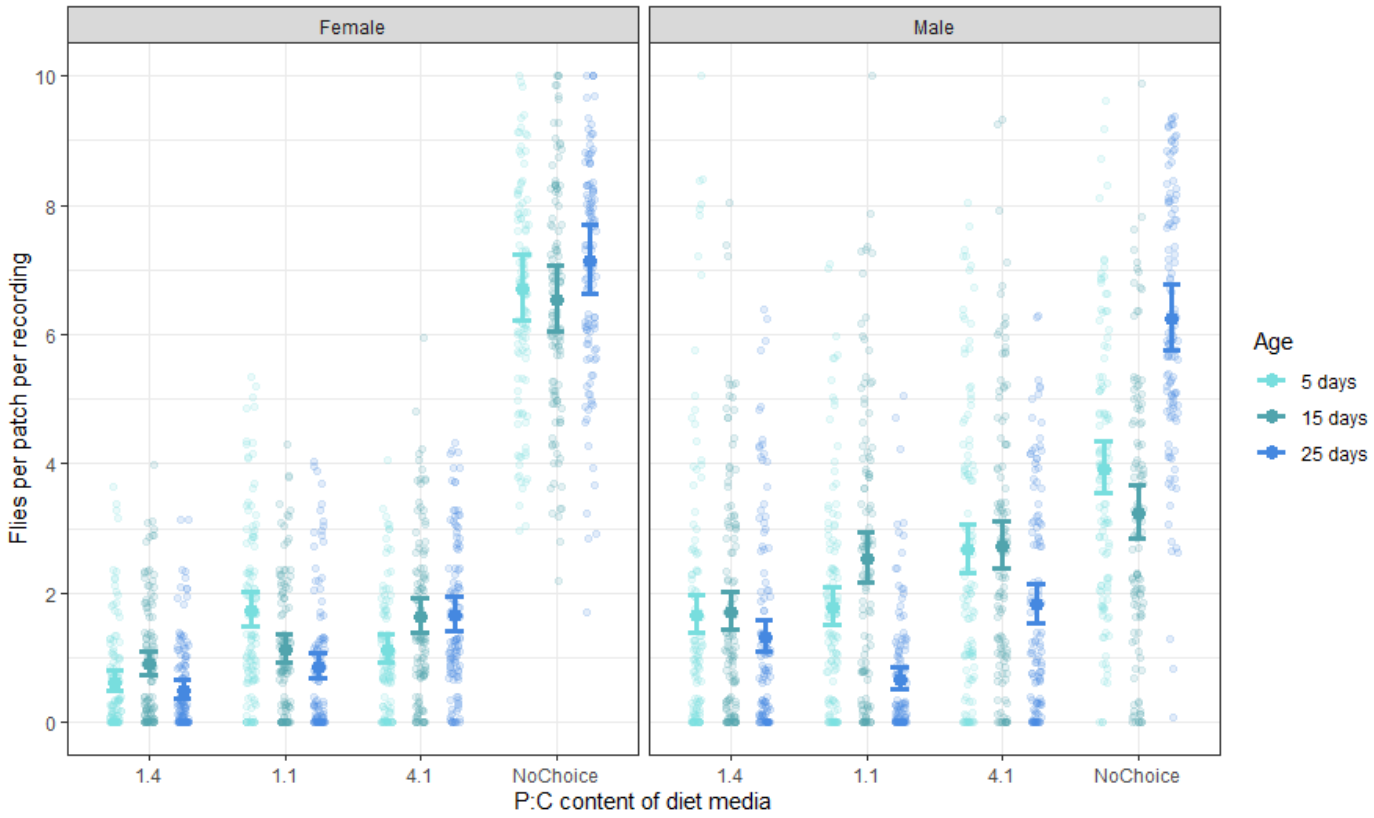

Figure 1: Diet preferences of 5, 15 and 25d females (left, N = 150/ age group) and males (right, N = 150/ age group) for 1:4, 1:1, 4:1 (P:C) or no diet patches. Shown is number of flies per diet patch per observation (8 x 30min intervals sampled per replicate, N = 15/ sex/ age group) for each of the diet patches. 'NoChoice' refers to individuals not on any diet patch at the time of observation. Points show estimated marginal means and error bars represent 95% asymptotic (Wald-type) confidence intervals calculated in emmeans (Lenth, 2023).

Age-dependent oviposition site selection

As for the dietary choice assays reported above, there was a significant interaction between the nutritional content of each diet patch and female age on the proportion of eggs laid per diet patch (X^2 = 88.0, df = 4, p = <0.0001; Table S8).

Pairwise comparisons (Table S17) showed that 25d old females laid proportionately more eggs on the 1:4 diet patch (*15d – 25d* estimate = -1.60, p <0.0001; *25d – 5d* estimate = 1.76, p <0.0001) and fewer eggs on the 1:1 diet (*15d – 25d* estimate = 1.41, p <0.0001; *25d – 5d* estimate = -1.33, p <0.0001) than did 5 or 15d females, while all age groups laid a consistent, smaller proportion of eggs on the 4:1 diet patch (*15d – 25d* estimate = -0.32, p = 0.60; *15d – 5d* estimate = -0.56, p = 0.18; *25d – 15d* estimate = -0.24, p = 0.69). Prediction #3 was therefore partially supported in that oviposition site selection was age-dependent, but the direction of these changes was not anticipated (Figure 2).

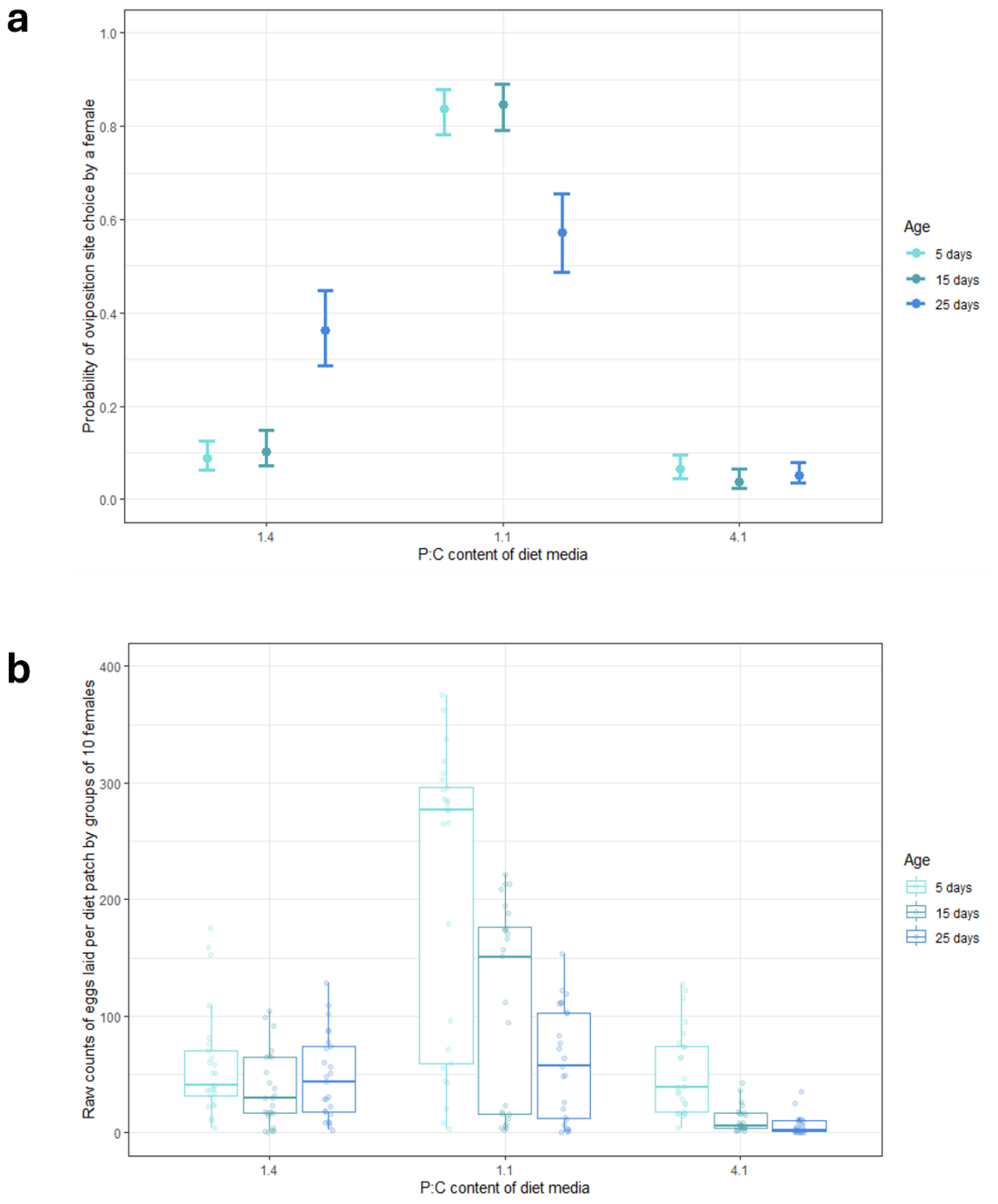

Figure 2: Oviposition site selection by 5, 15 and 25d females (N = 150 individuals/age group). A: the probability that females from each age group will deposit an egg on the three P:C patch options. Points show estimated marginal mean probabilities of oviposition site choice for each female age group derived from the beta-binomial GLMM. Error bars represent 95% asymptotic (Wald-type) confidence intervals calculated in emmeans (Lenth, 2023). **B:** Raw counts of eggs deposited on each patch by groups of 10 females.

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Larvae of older parents prefer a balanced P:C diet

Nutritional content of the diet patches significantly predicted larval dietary choice (X^2 = 48.0, df = 2, p = <0.0001). Surprisingly, there was a significant interaction between the nutritional content of diet patches and parental age in determining diet choice by larvae (X^2 = 11.49, df = 4, p = <0.02). Parental age alone had no effect on the total number of larvae recovered (X^2 = 0.27, df = 2, p = 0.87; Table S9).

Pairwise comparisons between parental age groups (Table S18) showed that the predicted preference of larvae for the 1:1 P:C diet (prediction #4) became apparent only at older parental ages. 1:1 was favoured above 1:4 by larvae of 15d parents (*1:1 – 1:4* estimate = 1.41, p <0.0001) and larvae of 25d parents (*1:1 – 1:4* estimate = 1.05, p <0.001) but not larvae of 5d parents (*1:1 – 1:4* estimate = 0.50, p = 0.07). Larvae of 15d parents also showed a preference for 1:1 over 4:1 (*1:1 – 4:1* estimate = 0.85, p <0.001) which was absent in larvae of 5d or 25d parents, while larvae of 25d parents showed a preference for 4:1 over 1:4 which was absent in the 5d and 15d parent groups (*1:4 – 4:1* estimate = 0.83, p <0.001). To summarise, larvae of 5d parents did not show significant preferences for any food patch over another; larvae of 15d parents preferred 1:1 over both 1:4 and 4:1, showing the expected pattern, with no difference in preference between non-preferred 1:4 and 4:1; and larvae of 25d parents preferred 1:1 or 4:1 equally over 1:4 (Figure 3).

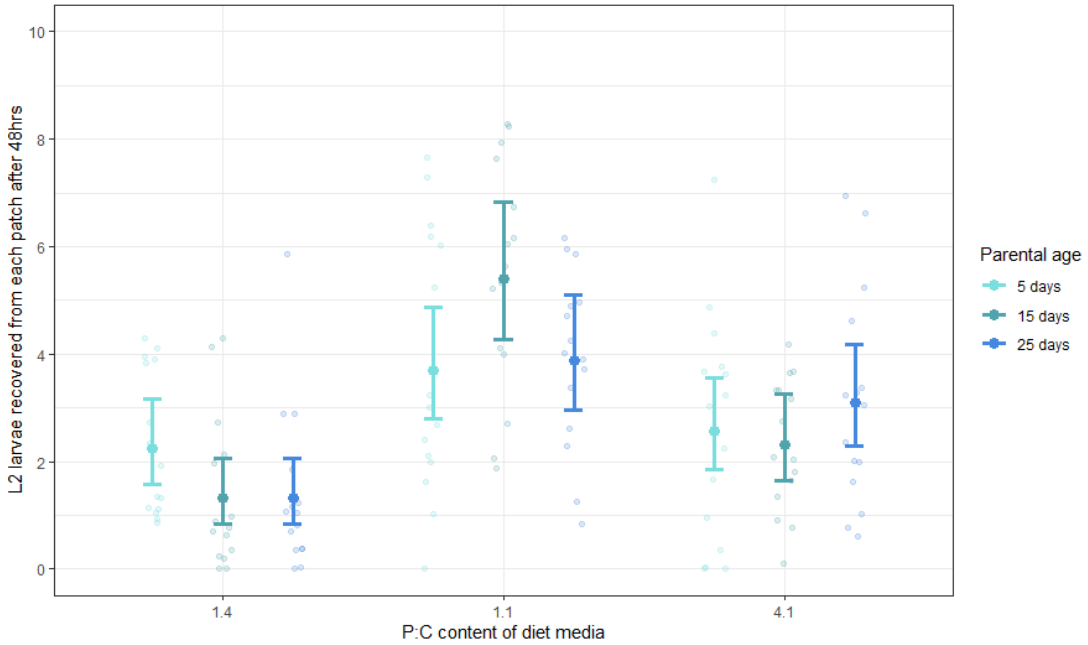

Figure 3: Number of larvae from 5, 15 and 25d old parents recovered from each diet patch after 48hr in choice assays (N = 150/ parental age group over 15 replicates/ parental age group). A total of 450 larvae were transferred to choice assays within 24hrs of hatching, of which 389 were recovered by manual extraction (equivalent to >8 out of the original 10 larvae per replicate, on average). Points show estimated marginal means and error bars represent 95% asymptotic (Wald-type) confidence intervals calculated in emmeans (Lenth, 2023).

Parental age, nutrition and their interaction determine offspring survival to adulthood

Parental age had a strong main effect on the probability that offspring would eclose within 14 days of development, with offspring of 5d old parents possessing a significant survival advantage over offspring of 15d old parents ($X^2 = 64.9$, df = 1, $p < 0.0001$). Rearing diet also significantly predicted eclosion success in both parental age groups ($X^2 = 15.8$, df = 2, $p < 0.001$). The interaction of parental age and rearing diet was also a significant determinant of offspring eclosion success ($X^2 = 41.6$, df = 2, $p < 0.0001$; Table S11-12). We note that offspring of 15d parents began to eclose two full days later than offspring of 5d parents on all diets (Figure 4).

Pairwise comparisons in post-hoc Tukey tests (Table S19-20) showed that eclosion probability was equal among diet groups in offspring with 5d parents, despite the prediction of protein toxicity on the 4:1 diet (*1:1 – 1:4* estimate = 0.50, p = 0.36; *1:1 – 4:1* estimate = 0.01, p = 1.00; *1:4 – 4:1* estimate = -0.49, p = 0.38). This was not the case in offspring with 15d parents, in which survival was significantly higher on 1:4 than 1:1 (*1:1 – 1:4* estimate = -0.99, p = 0.01) or on 4:1 (*1:4 – 4:1* estimate = 3.20, p <0.0001) and higher on 1:1 than on 4:1 (*1:1 – 4:1* estimate = 2.21, p <0.0001).

Due to low survivorship of individuals reared on 4:1 with 15d parents, time to eclosion could only be analysed in the 1:4 and 1:1 diet groups. Results showed that larvae with 5d parents reached eclosion faster than larvae with 15d parents (SI 1.1). The described patterns of preadult mortality may also have given rise to observed diet and parental age-dependent differences in morphology in adult offspring via selective disappearance (SI 1.2-1.3).

Prediction #5 was that larval survival would be highest on the 1:1 P:C diet, and we did not predict a main effect of parental age. Our results contradicted this in that the probability of eclosion by 14 days in 5d parent larvae was not affected by rearing diet, and in the 15d parent group, probability of eclosion by 14 days was highest on the 1:4 diet despite developmental delay (SI 1.1).

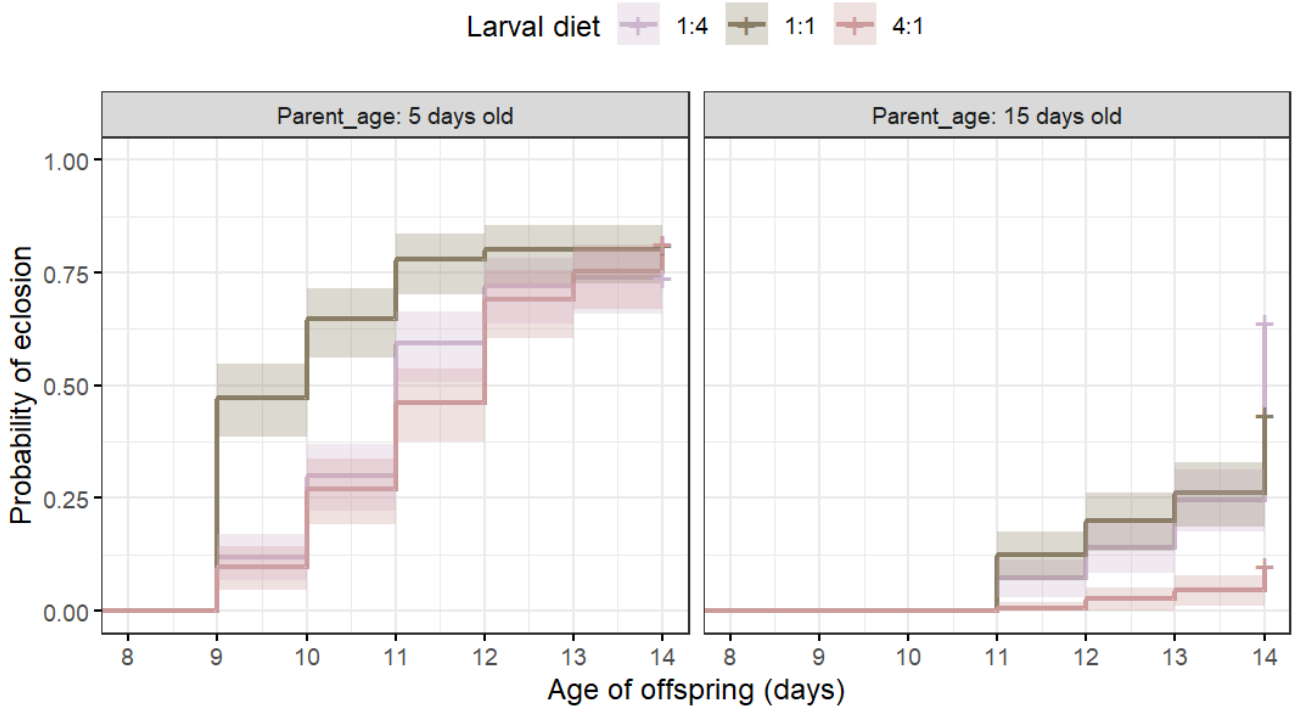

Figure 4: Probability that larvae reared on each of the three experimental diets under no-choice (N = 150 per parental age group per diet) would emerge from their pupae within 14 days when parents were 5d (left) versus 15d (right). Shown are Kaplan-Meier time-to-event curves based on time to eclosion in individual offspring which successfully eclosed (N = 523). As peak eclosion usually occurs at approximately 9 days, individuals which did not eclose by 14 days are likely to have died (N = 377).

Discussion

Here we tested for sex-, age- and lifestage-specific differences in diet preferences, and investigated how oviposition site choices of mothers of different ages influence offspring fitness. The results showed significant effects of age and sex on diet patch selection, and that oviposition site selection in females was age-sensitive and only partially aligned with the diet choices made by larvae. Unexpectedly, we also found that a modest increase in parental age (from 5 to 15d) led to increased preadult mortality in offspring. Preadult survival of offspring of 5d parents was not significantly influenced by nutrition, while in offspring of 15d parents, preadult survival had a strong relationship to dietary protein, with preadult survival maximised when protein was low.

Diet choice was sex-and-age specific, but did not follow predicted patterns

We predicted that females would favour high protein diets when young, and that this preference would diminish with age as their fertility declined. Males of all ages were expected to consistently favour high carbohydrate diets. Instead, diet preference varied with age in unexpected ways in both sexes.

Males showed changing patterns of both patch occupancy (the choice to stand on any patch versus the dish) and diet patch choice with age. 25d males favoured high protein and avoided the 1:1 balanced diet patch more than did 15d or 5d males, while preference for high carbohydrate diet patches remained consistent over age. One potential explanation is that males of all ages visited high carbohydrate patches to feed while choosing to gather on other diet patches in the expectation that these were sites were more likely to attract females. Also contrary to predictions, 5d females did not prefer high protein diets in comparison to 15d or 25 d females. Instead, the oldest (25d) females visited the 4:1 diet more than both groups of younger females. It could be that multiple matings over a female's lifetime gradually increase protein appetite (Camus et al., 2018; Sydney et al., 2024). Counter to this idea, 15d females visited the 1:4 diet more often than younger or older females. Overall, the results showed that the relationship between female age and dietary preference is non-linear.

We quantified presence on a diet patch rather than quantifying diet consumed. Therefore, sex and age-specific patterns we observed in patch occupancy (the choice to be present on a specific diet patch versus on the dish) could represent differences in social behaviour around resources in addition to indicating patterns of nutrient intake. In all age groups, females were more likely to be observed on the dish rather than on a patch. In contrast, younger males (5 and 15d) tended to congregate on patches for the duration of the assay. Male *D. melanogaster* are known to consume less food overall than females (Camus et al., 2018). Therefore, rather than feeding, it is possible that 5 and 15d males gathered on the food patches in anticipation of mating opportunities with females. Older (25d) males did not show this pattern and instead more closely resembled females in being more frequently observed standing on the dish. This may reflect a reduction in competitive behaviour, perhaps arising from increased frailty or sperm depletion from multiple matings throughout the life course. Male diet choice was insensitive to mating rates in a recent study in which matings were controlled and occurred within a single day (Sydney et al., 2024). In the present study, males were co-housed with age-matched females under a 1:1 sex ratio throughout their lifetimes. The observed switch in patch choice behaviour could therefore have been driven by the presence of same-sex competitors combined with age-related decline.

Oviposition site selection changed direction in older females

Ovipositing females are known to continuously assess their surroundings and alter their egg laying preferences according to the types of substrates available (Vijayan et al., 2022). We had hypothesised that oviposition site selection would become more variable as females aged, since selection acting to maintain advantageous egg laying behaviours weakens after the onset of reproduction, as predicted by classical theories of ageing (Hamilton, 1966; Kirkwood & Holliday, 1979; Meyer et al., 2025; Rose, 1994). However, rather than showing weaker preference, the oldest females laid a larger proportion of their eggs on the sucrose-biased 1:4 patch and fewer on the balanced 1:1 patch favoured by the younger age groups.

Sucrose aversion by ovipositing females is widely reported and has been exploited as an experimental paradigm by which to model simple decision-making (Chen et al., 2022; Otarola-Jimenez et al., 2025; Vijayan et al., 2022; Yang et al., 2015). Larvae are attracted to intermediate concentrations of sucrose, but repelled by very high concentrations (Schipanski et al., 2008). Interestingly, females' aversion to ovipositing on sucrose has been reversed by activating dopaminergic neurons (C.H. Yang et al., 2015). Oviposition descending neurons have also been implicated in sucrose avoidance by ovipositing females (Vijayan et al., 2023). If these pathways are subject to age-related changes, this could provide a possible mechanism to explain older females' increased acceptance of high sucrose media. Future work employing sucrose-based oviposition assays might benefit from accounting for female age as a confounding or explanatory variable.

The 5d females preferred to lay eggs on the 1:1 diet patch but, unlike older cohorts, did not show any additional preference between the nonpreferred 1:4 and 4:1 patches. The higher fecundity of the youngest females might mitigate potential costs of placing eggs on suboptimal substrates and therefore allow for less selectivity. Alternatively, it may indicate that less preferred patches become increasingly acceptable as favoured sites become overloaded with other females' eggs, thus minimising competition and density dependence, which may have been less intense in the less fecund 15 and 25d cohorts. Future work could use video capture to record density-dependent changes in decision-making.

As larvae cannot fly to a more optimal patch after hatching, oviposition site choice predetermines the nutritional environment experienced by larvae for the duration of their preadult development. In this way, changes in oviposition site choice by ageing females can be considered a form of behaviourally-driven intergenerational maternal age effect. Such effects usually refer to epigenetic modifications, but non-genetic intergenerational effects can also encompass provisioned nutrients and transmitted behaviours (Bonduriansky et al., 2012; Bonduriansky & Day, 2009). Viewed through this lens, age-dependent

changes in oviposition site preference could represent an underappreciated mechanism by which maternal age can shape offspring outcomes in insects.

Larvae choose a balanced diet, unless they have very young parents

Over recent decades, nongenetic parental effects have gained recognition as a significant source of variation in offspring phenotypes. Key mechanisms involved include DNA methylation and histone modifications (Greer et al., 2011) and, in fathers, the age-related accumulation of de novo mutations (Kong et al., 2012). Despite these major steps forward, the taxonomic generality (Ivimey-Cook et al., 2023) and evolutionary significance of such parental effects have not yet been fully evaluated. Differences or imbalances in the baseline condition of newly hatched older-parent larvae could promote more specific intake trajectories as larvae attempt to compensate, explaining the more targeted feeding shown by older-parent larvae in choice assays. In prediction #3 we outlined that, under dietary choice, larvae might consistently favour the 1:1 diet patch as balanced macronutrient intake is known to shorten larval development time (Gray et al., 2018; Rodrigues et al., 2015). This expectation was largely met, but larvae of the youngest parents were more evenly distributed throughout the diet patches than those of older parents, perhaps signifying a greater tendency towards exploratory foraging behaviour in this group.

Larval development followed expected patterns while also responding strongly to parental age

To evaluate the diet choices made by larvae and by ovipositing females, we reared additional larvae from the 5 and 15d parental cohorts in vials of 1:4, 1:1 or 4:1 diet. Importantly, in this experiment, offspring were transferred onto experimental diets only after hatching. Differences in larval survival therefore reflect intrinsic characteristics of the larvae rather than parent fertility. These effects were unexpected given the relatively modest difference in parental age between cohorts (5 versus 15d). This result could indicate that even early reproductive ageing may carry consequences for offspring performance when exposed to suboptimal environments.

As parents were drawn from a freely mating, co-aged population, it is possible that constant availability of matings caused a decline in gamete or ejaculate quality, affecting offspring phenotypes. However, recent work demonstrates that paternal sexual rest influences offspring phenotypes independently of paternal age, with the potential to inflate age-related paternal effects (Sanghvi, Gascoigne, et al., 2025), and that seminal fluid in older or multiply mated males is a stronger determinant of offspring number than sperm quantity (Sanghvi, Shandilya, et al., 2025). In this freely mating population with an even sex ratio, sperm storage occurring from paternal sexual rest is unlikely to have contributed to the observed decline in offspring quality. On the maternal side, provisioning of eggs is a well-documented route by

which maternal age can influence offspring outcomes in invertebrates. This could perhaps extend to or resemble maternal exhaustion within the multiply mating mixed population in this study. In the parasitoid wasp *Eupelmus vuilletti*, older mothers provision eggs with less protein and sugar (Giron & Casas, 2003; Muller et al., 2017). This deficit partially closes after hatching, possibly facilitated by the parasitic ecology of this species, however differences in glycogen dynamics persist into adulthood (Muller et al., 2017). If the 15d mothers in the present study provisioned eggs with fewer or less balanced resources than 5d mothers, this could account for the differences in larval performance across experimental diets.

Differences in provisioning quality may also explain the disparities in morphological outcomes observed across parent age and diet groups (SI 1.2 – 1.3), however, selective disappearance is another viable explanation for these differences given the high preadult mortality in some treatment groups. While the survival of older-parent offspring was reduced among all diet treatments here, shifts in parental provisioning may not always reduce viability or fitness. In the orb-weaving spider *Argiope radon*, older mothers produce fewer, heavier offspring which develop more slowly but exhibit greater starvation resistance compared to the more numerous, faster-developing offspring of younger mothers (Ameri et al., 2019). It is possible that older parents, especially mothers, 'recalibrate' investment adaptively as their reproductive output declines, in a manner which confers advantage under some environmental conditions. In choice assays, older-parent larvae showed no specific avoidance of the 4:1 diet patch despite its apparent toxicity under no-choice conditions, while older mothers preferentially deposited eggs on the 1:4 substrate which yielded the highest survivorship among older-parent offspring in no-choice assays. This raises the possibility that older mothers may have been exercising 'damage control' by adaptively modifying their oviposition site selection to align with the most optimal patch for their differently provisioned offspring.

Summary and conclusions

This study characterised how the ageing process interacts with nutritional ecology across multiple life history stages by integrating sex-and-age specific dietary choice, oviposition site selection, and offspring development outcomes within a unified framework. Male and female dietary trajectories responded in a sex-and-age dependent manner, and oviposition site selection also changed significantly with age. Most strikingly, a modest increase in parental age exerted strong intergenerational effects on offspring responses to dietary manipulation. These manifested across survival, development time and adult morphology, highlighting an important and potentially underappreciated role for the interaction between parental effects and the early nutritional environment in shaping offspring life history.

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638 https://doi.org/10.1111/jeb.12872

Supplementary information

1. Supplementary results

1.1 Parental age and diet determined larval development time under no-choice rearing conditions (1:4 and 1:1 diets only)

Parental age had a significant main effect on time to eclosion in both the 1:4 and 1:1 diets (X^2 = 321.5, df = 1, p <0.0001). Rearing diet also had a significant effect on development time (X^2 = 22.6, df = 1, p < 0.0001). The interaction of parental age and rearing diet was marginally nonsignificant (X^2 = 3.51, df = 1, p = 0.06; Table S11).

Pairwise comparisons (Tables S21-S22) showed that larvae of 5d parents reached eclosion faster than larvae of 15d parents on both diets (1:4 reared larvae: *15d parents – 5d parents* estimate = 0.22, p <0.0001; 1:1 reared larvae: *15d parents – 5d parents* estimate = 0.27, p <0.0001). Larvae of 5d parents showed faster development on 1:1 than on 1:4 , as expected in prediction #5 (*1:1 – 1:4* estimate = -0.09, p <0.0001). However, this effect was marginally nonsignificant in larvae of 15d parents (*1:1 – 1:4* estimate = -0.04, p = 0.06).

1.2 Parental age interacts with nutrition to determine offspring wing and thorax size

Wing length (Figure S3a) was determined by a main effect of sex, as expected (X^2 = 509, df = 1, p <0.0001), a main effect of rearing diet (X^2 = 22.1, df = 2, p <0.0001) and an interaction between rearing diet and parental age (X^2 = 103, df = 2, p <0.0001; Table S14).

Pairwise comparisons (Table S23) showed that wing size in males and females responded similarly to diet and parental age, remaining proportionate to the size difference between the sexes. In individuals with 15d parents, wing size was smaller than in individuals with 5d parents when reared on 1:1 (Males:

estimate = -0.17, p <0.0001; Females: estimate -0.23, p <0.0001), but larger than in individuals with 5d parents when reared on 1:4 (Males: estimate 0.12, p <0.001; Females: estimate 0.14, p <0.0001). A sex difference emerged in wing length responses to 4:1 by parental age in that sons of 15d parents had significantly longer wings than sons of 5d parents (estimate = 0.13, p = 0.001) while this effect was marginally nonsignificant in daughters (estimate = 0.08, p = 0.08).

Thorax length (Figure S3b) showed the expected strong effect for sex (X^2 = 496, df = 1, p <0.0001) and a main effect of rearing diet (X^2 = 14.5, df = 2, p <0.001), and an interaction between rearing diet and parental age (X^2 = 7.55, df = 2, p = 0.02; Table S13).

Pairwise comparisons (Table S24) revealed that patterns of thorax size did not completely match wing length leading to differences in proportionality by treatment group (SI 1.3). Females reared on 1:1 with 15d parents had smaller thoraxes than females reared on 1:1 with 5d parents (estimate = -0.04, p = 0.04) while there was no parental age difference in thorax size in individuals reared on 1:4 (estimate = 0.02, p = 0.24) or 4:1 (estimate = -0.03, p = 0.33). Meanwhile, males with 15d parents reared on 1:4 had larger thoraxes than males with 5d parents reared on 1:4 (estimate = 0.06, p <0.01). This was marginally nonsignificant in males reared on 4:1 (estimate = 0.05, p = 0.06) and absent in males reared on 1:1 (estimate = -0.002, p = 0.92).

1.3 Parental age and rearing diet exert sex-specific effects on offspring body shape

Like the analyses of wing and thorax length, there was a significant effect for the interaction of parental age and rearing diet (X^2 = 10.2, df = 2, p <0.01). A marginal effect was also detected for an interaction of sex by parental age in determining wing/ thorax proportionality (X^2 = 3.77, df = 1, p = 0.051; Table S15).

Sexual dimorphism was also detected in the ratio of wing length to thorax length; males had longer wings relative to their thoraxes, and females had shorter wings relative to their thoraxes (X^2 = 47.7, df = 1, p < 0.0001).

Pairwise comparisons (Table S25) in post-hoc Tukey tests showed more varied responses in females than in males (Figure S4). Both male and female offspring of 15d parents reared on 1:1 had smaller wings/ larger thoraxes than offspring of 5d parents (Males: estimate -0.19, p = 0.03; Females: estimate -0.15, p = 0.02), while female offspring of 15d parents reared on 4:1 had larger wings/ smaller thoraxes than female offspring of 5d parents reared on 4:1 (estimate = 0.18, p = 0.03).

2. Supplementary methods

2.1 Body size measures

Frozen offspring vials were removed from the freezer in batches and every individual (N = 523) was photographed using an Eagle-M microscope with a GXCAm screen. Photographs were taken in lateral view which provides clear visibility of the thorax and wings (Figure S2). All measurements were taken in imageJ v1.54i. To analyse the effects of rearing diet and parental age on body proportions, wing length was divided by thorax length for each individual to obtain a measurement of proportionality. Individuals with damaged or awkwardly positioned wings were included in thorax analyses but excluded from wing length and proportionality analyses (N = 8).

Supplementary figures

Figure S1

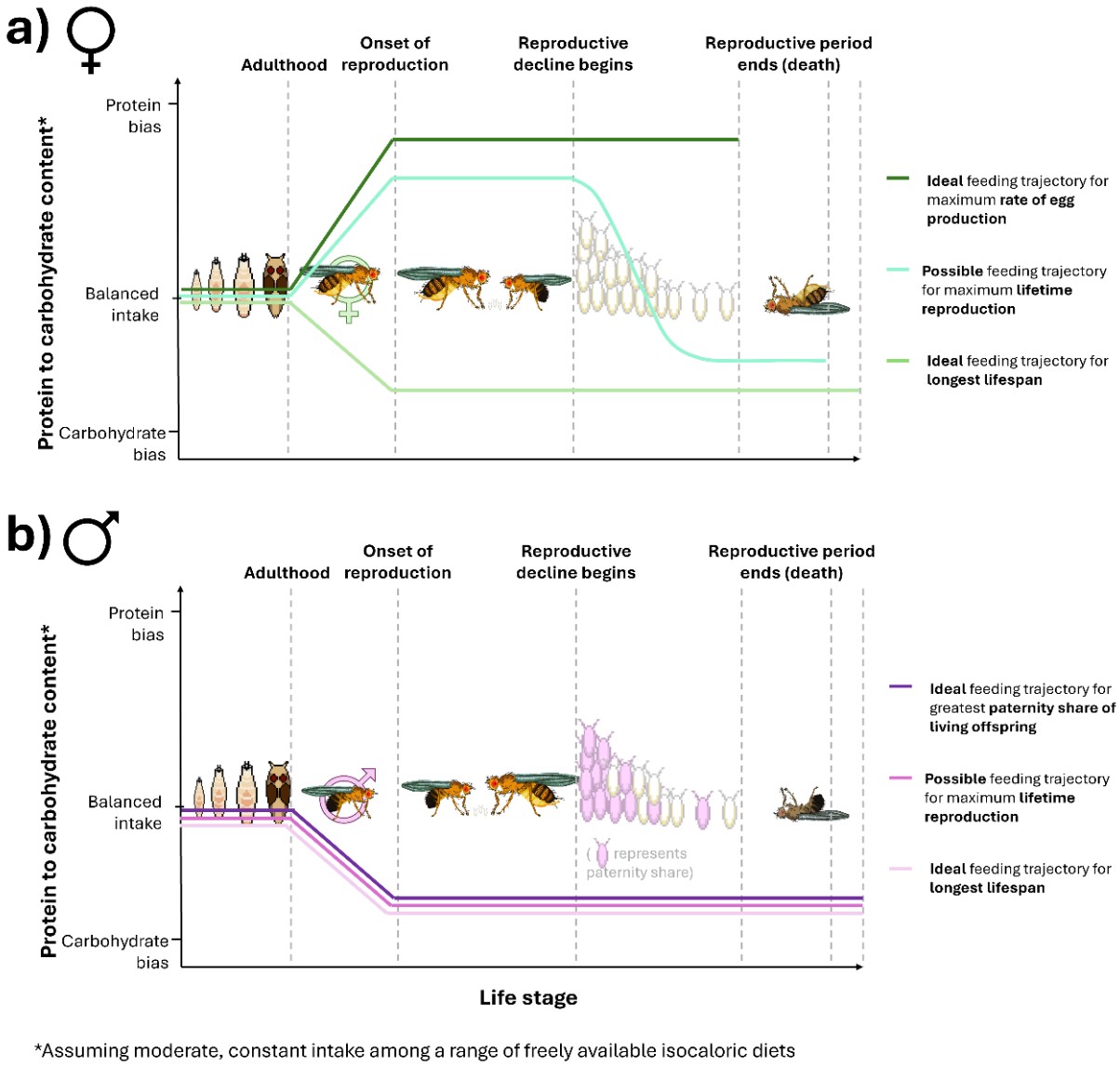

Figure S1: A schematic visualisation comparing how each sex might adjust feeding trajectory over the life course. For simplicity, this assumes constant availability of a range of isocaloric food sources over a gradient of high protein to high carbohydrate content. **a**: Hypothesised intake trajectories which could be taken by females. The **dark green/top** trajectory would maximise the rate of egg production and result in shorter lifespan; the **blue-green/middle** trajectory could support both high egg production in early life and extend reproductive lifespan, potentially leading to maximised lifetime reproduction; lastly, the male-like **pale green/bottom** trajectory would maximise lifespan at cost to reproduction. **b**: Hypothesised intake trajectories which could be taken by males. Unlike females, the same high-carbohydrate and low-protein trajectory is predicted to benefit short-term reproductive rate, understood here as paternity share of eggs at any given time (**purple/top**), lifetime reproductive output (**hot pink/middle)**, and lifespan (**light pink/bottom)**.

Figure S2

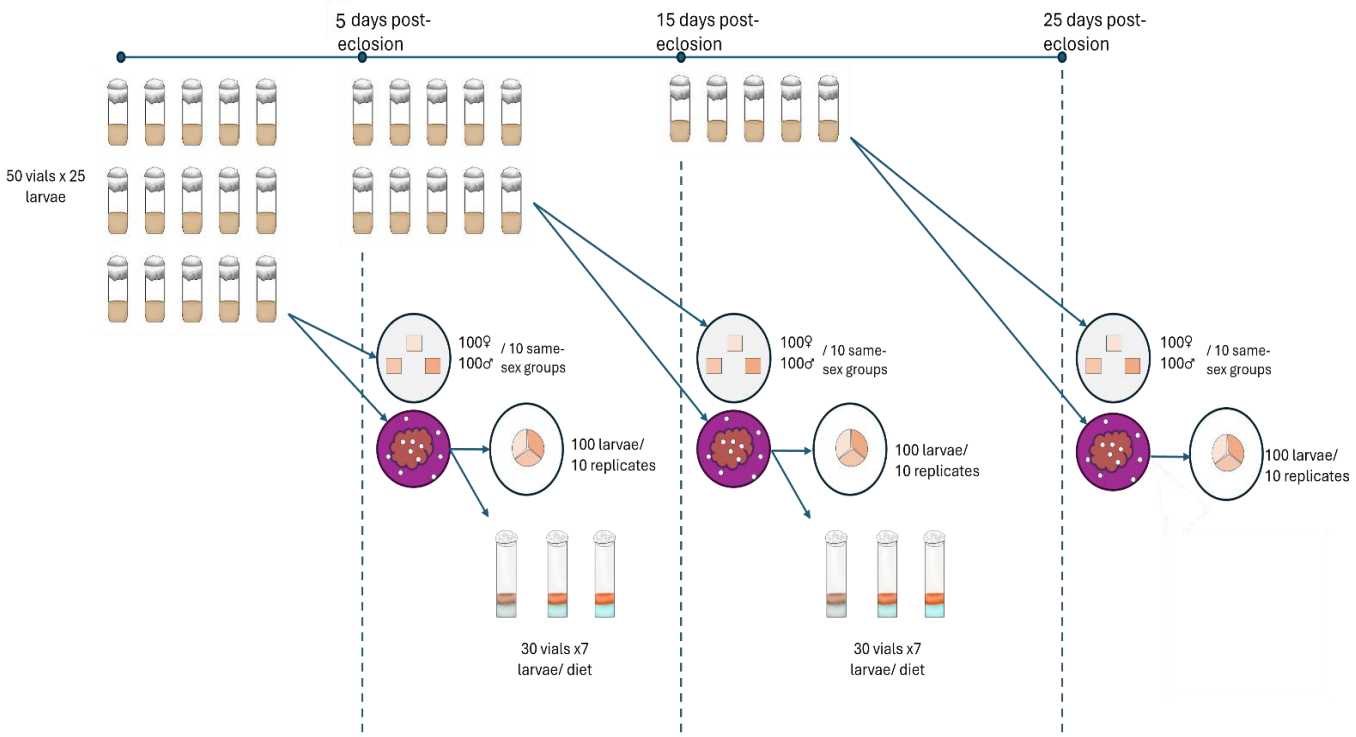

Figure S2: **Schematic illustrating how individuals were sampled at three time-points from a large, co-aged population for assays of adult dietary choice, larval dietary choice and larval development under no-choice.** We first established a large, low density cohort of standardised parental age by transferring larvae of 5 day old parents to 50 vials of SYA in groups of 25 (N = 1,250). After eclosion, individuals were transferred to fresh SYA vials in groups of 10 males and 10 females (N = 1,200), and thereafter were transferred to fresh media twice weekly until the end of the experiment. At 5d post eclosion, one third of this cohort (N = 400 individuals in 20 vials) were selected at random and split into two groups. One group (N = 200) was used for adult diet choice assays, while eggs were collected from the other group (N = 200) over a 24hr period on plates of purple grape juice agar. Eggs from the latter group were incubated for a further 24hrs, and then first instar larvae were collected for larval choice assays and assays of larval development under no-choice on the 1:4, 1:1 and 4:1 experimental diets. At 15d post eclosion, a further 400 individuals were randomly sampled from the large starting cohort, split into two groups of 200 individuals, and these groups were used again for either adult choice assays or egg collection for larval diet choice and larval non-choice development assays. At 25d post eclosion, the remaining individuals (approximately 380) were again assigned at random to the diet choice group

Figure S3

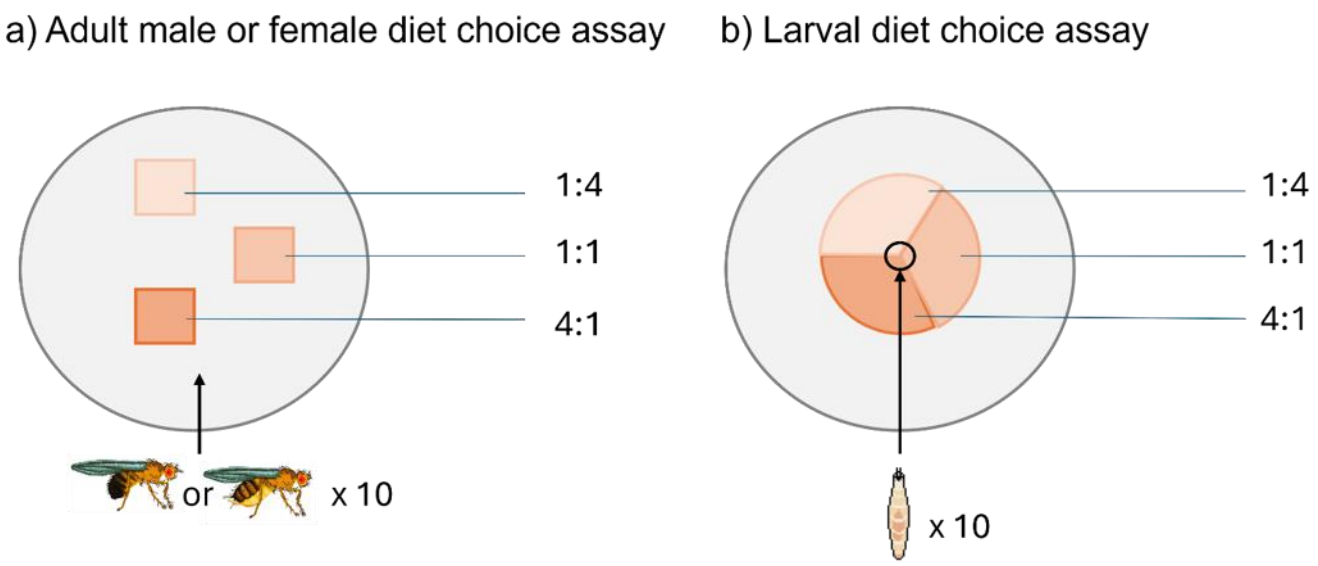

Figure S3: **Illustration showing how three-choice assays were configured for adult males or females (a) and for larvae (b).** These utilised synthetic solid diets with macronutrient concentrations at 1:4, 1:1 and 4:1 parts protein (casein) to carbohydrate (sucrose). **A**: Choice assay setup used for single-sex groups of 10 adult males or females at 5, 15 and 25d after eclosion. Flies were introduced to the plates under light CO_2 sedation and allowed 1h to recover and acclimatise, after which we recorded the number of flies gathered on each diet patch or on no patch (any individuals standing on the sides or edges of the plate, not touching any diet patch) at half-hourly intervals over a period of 4h. **B**: Choice assay setup used for larvae. Eggs were collected from the parental cohort at 5, 15, and 25d post eclosion on plates of purple grape juice agar. After hatching, groups of 10 first instar larvae were collected from the purple grape plates and placed at the centre of the choice assay plates where the three diet patches intersect. After a period of 48hrs of free foraging, the diet patches were pulled apart and the larvae within each patch were manually extracted and counted to assess diet choice.

Figure S4

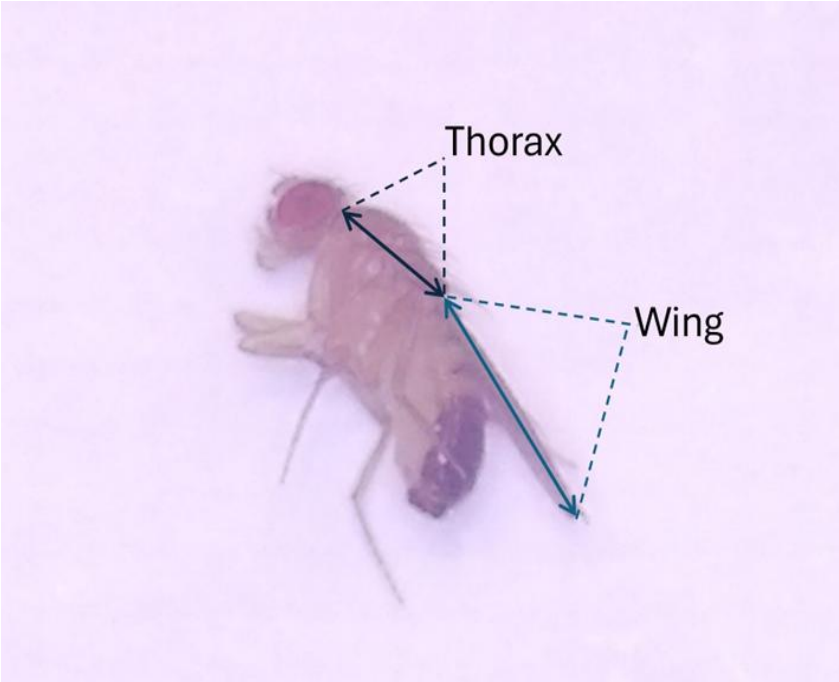

Figure S4: **An example photograph from the dataset for wing and thorax measures of the eclosed offspring from the non-choice development experiment, with markers illustrating the measurement points.** Thorax length (in mm) was measured from the back of the head to the 'ridge' at the end of the thorax which is well-defined in lateral view. Wing measurements were taken from this point to the end of the closest wing, and only when wings were undamaged and held in the folded position pictured.

Figure S5

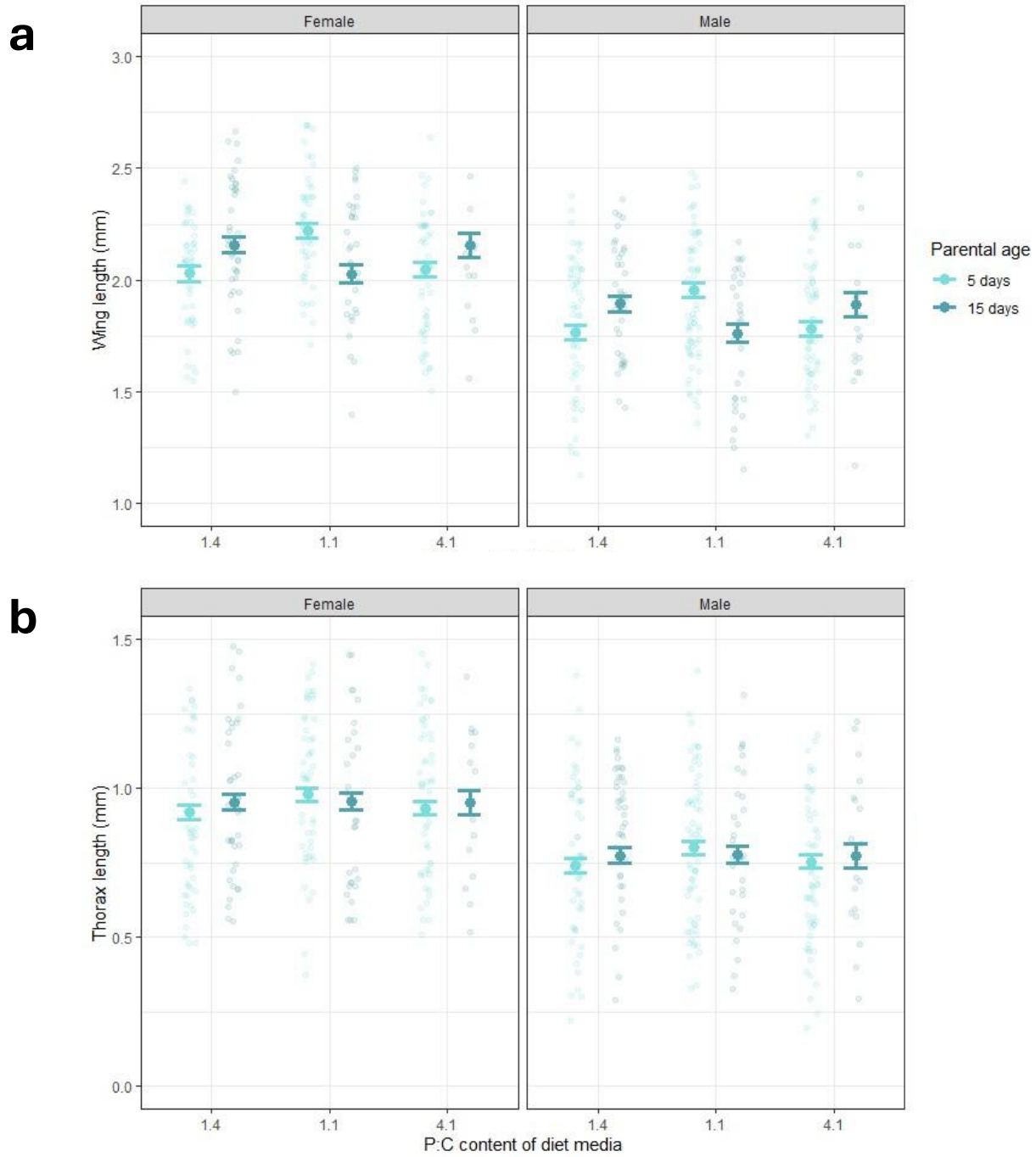

Figure S5: A: wing length and B: thorax length in male and female offspring of 5d parents (N = 341) versus 15d parents (N = 182) when reared under no-choice on the three experimental diets. a: Shown is wing length in mm measured from the base to the tip of the wing in lateral view for each individual (Figure S2). Offspring of 5d parents had longer wings than offspring of 15d parents if offspring were raised on the 1:1 P:C diet, but smaller wings than 15d parents if offspring were raised on 1:4 or 4:1 P:C diets. This pattern was present in both sexes. **b:** Shown is thorax length in mm measured from the back of the head to the base of the wing in lateral view (Figure S2). In females, there was no difference in thorax size by parental age. There was no difference in thorax size by parental age in male offspring reared on the 1:1 P:C diet, but male offspring of 5d parents reared on the 1:4 or 4:1 P:C diets had smaller thoraxes than male offspring of 15d parents. Taken together, these wing and thorax size differences led to differences in proportionality among parental age and offspring rearing diet groups (SI 3.3). Points show estimated marginal means and error bars represent 95% asymptotic (Wald-type) confidence intervals calculated in emmeans (Lenth, 2023).

Figure S6

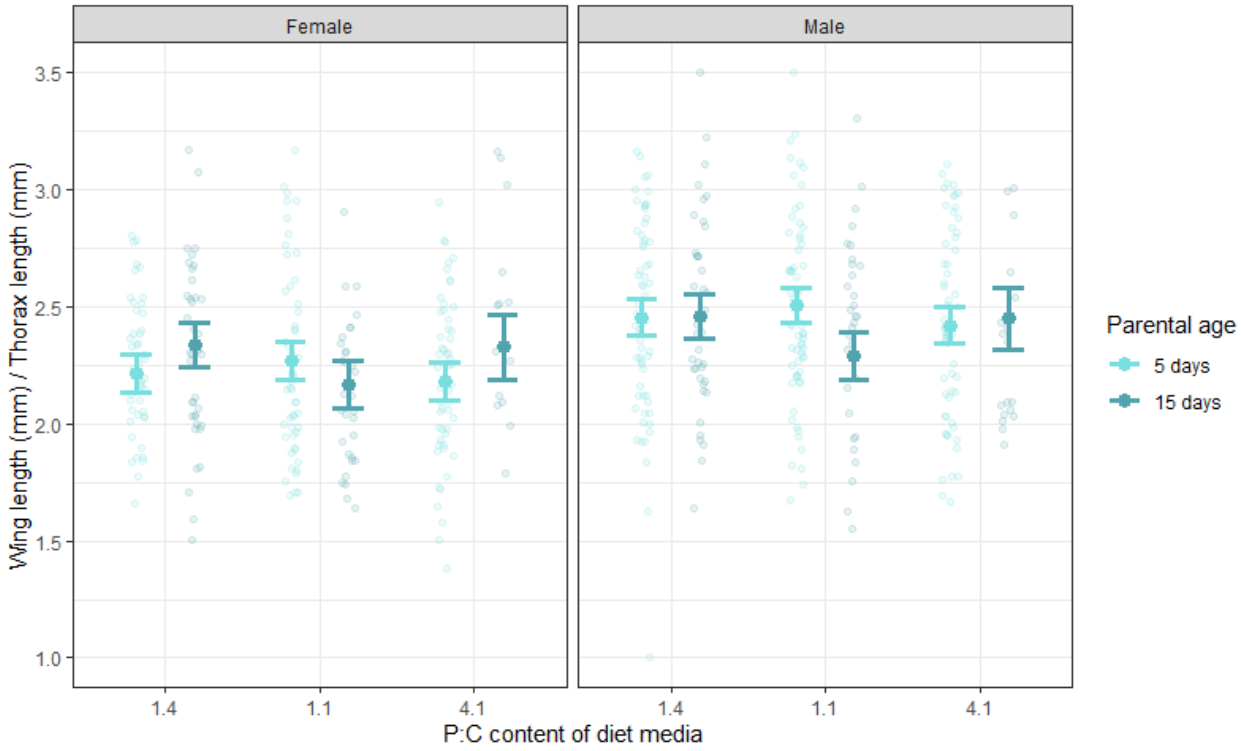

Figure S6: Wing length (mm) divided by thorax length (mm) in offspring of 5d (N = 341) versus 15d parents (N = 182) reared on the three experimental diets. Larger values indicate longer wings relative to thoraxes. In both males and females, offspring of 15d parents reared on 1:1 has smaller wings and larger thoraxes relative to offspring of 5d parents reared on 1:1. In addition, female offspring of 15d parents reared on 4:1 had larger wings and smaller thoraxes than female offspring of 5d parents reared on 4:1. Points show estimated marginal means and error bars represent 95% asymptotic (Wald-type) confidence intervals calculated in emmeans (Lenth, 2023).

3. Supplementary tables

4.1 Meridic diet recipes

Table S1: Recipe for 1L each of the sucrose-casein meridic diets

Ingredient	**1:4**	**1:1**	**4:1**
Casein(g)	24	60	96
Sucrose (g)	96	60	24
Agar(g)	100	100	100
Cholesterol(g)	100	100	100
Lecithin(g)	100	100	100
K2HPO4(ml)	100	100	100
KH2PO4(ml)	100	100	100
MgSO4(ml)	100	100	100
MaHCO3(ml)	100	100	100
Nucleic acid(ml)	100	100	100
Deioinised water(ml)	200	200	200
Vitamin mix(ml)	150	150	150
Nipagin(ml)	10.0	10.0	10.0
Propionic acid(ml)	3.00	3.00	3.00

Table S2: Recipe for 1L nucleic acid solution used in experimental diets

Ingredient	**Quantity**
Uridine(g)	5.70
Inosine(g)	6.40
Deinonised water	1000

Table S3: Recipe for 1L vitamin mix solution used in experimental diets

Ingredient	**Quantity**
Thiamine(g)	0.013
Riboflavin(g)	0.066
Nicotinic acid(g)	0.800
Calcium pantothenate(g)	0.111
Pyridoxine(g)	0.016
Biotin solution(ml)	66.50
Folic acid solution(ml)	66.50
Deionised water(ml)	866.5

Table S4: Recipe for 1L biotin solution used in vitamin mix

Ingredient	**Quantity**
Biotin(g)	0.02
Deionised water(ml)	1000

Table S5: Recipe for 1L folic acid solution used in vitamin mix

Ingredient	Quantity
Folic acid(g)	0.075
Ethanol(ml)	200
Deionised water(ml)	800

4.2 Model table

Table S6: Model families by level of individual and traits of interest

Model family	Level of individual	Trait of interest	Fixed effects	Random effects
Negative binomial	P0 (mated males and females)	Diet choice	Sex, age, diet patch	Replicate (plate of 10 individuals), observation time
Beta-binomial	P0 (mated females)	Oviposition site choice	Age, diet patch	Replicate (plate of 10 individuals), observation (per diet patch per plate)
Poisson	F1 (larvae)	Diet choice	Diet patch, parental age	Replicate (plate of 10 individuals), observation (per diet patch per plate)
Cox Proportional Hazards	F1 (larvae)	Egg-to-adult development time	Diet patch, parental age	Vial (of 5 individuals), individual ID
Conway-Maxwell Poisson	F1 (larvae)	Egg-to-adult development time	Diet patch, parental age	Vial (of 5 individuals), individual ID
Binomial	F1 (larvae)	Preadult mortality	Diet patch, parental age	Vial (of 5 individuals)
Gaussian	F1 (adults)	Thorax length	Sex, diet patch, parental age	Vial (of 5 individuals), individual ID
Gaussian	F1 (adults)	Wing length	Sex, diet patch, parental age	Vial (of 5 individuals), individual ID
Gaussian	F1 (adults)	Wing length/ thorax length (proportionality)	Sex, diet patch, parental age	Vial (of 5 individuals), individual ID

3.3 Full model outputs

Table S7

Table S7: Full model output for diet choice over the age course in mated males and females at 5, 15 and 25d. Shown here is the summary of the negative binomial generalised linear mixed effects model for all fixed effects. Random effects were included for replicate and observation time, and a zeroinflation parameter was used to model zeroinflation as a constant probability across all observations ("~1").

Predictor	**Estimate**	**Std. error**	**Z value**	**Pr (>|z|)**
(Intercept)	0.11935	0.09672	1.234	0.217213
Sex (Male)	0.81023	0.12148	6.670	2.56e-11 ***
Age (25d)	-0.25932	0.14646	-1.771	0.076626 .
Age (5d)	0.43498	0.12382	3.513	0.000443 ***
Patch (1:4)	-0.22882	0.14465	-1.582	0.113657
Patch (no patch)	1.75844	0.10459	16.812	<2e-16 ***
Patch (4:1)	0.37042	0.12548	2.952	0.003158 **
Sex (Male) * Age (25d)	-1.06486	0.20654	-5.156	2.53e-07 ***
Sex (Male) * Age (5d)	-0.78297	0.16300	-4.804	1.56e-06 ***
Sex (Male) * Patch (1:4)	-0.16957	0.18039	-0.940	0.347223
Sex (Male) * Patch (no patch)	-1.51734	0.13878	-10.933	<2e-16 ***
Sex (Male) * Patch (4:1)	-0.29690	0.15793	-1.880	0.060115 .
Age (25d) * Patch (1:4)	-0.32888	0.23149	-1.421	0.155407
Age (5d) * Patch (1:4)	-0.79283	0.20862	-3.800	0.000145 ***
Age (25d) * Patch (no patch)	0.34859	0.15636	2.229	0.025790 *
Age (5d) * Patch (no patch)	-0.40964	0.13574	-3.018	0.002546 **
Age (25d) * Patch (4:1)	0.27675	0.18514	1.495	0.134970
Age (5d) * Patch (4:1)	-0.79989	0.17576	-4.551	5.34e-06 ***
Sex (Male) * Age (25d) * Patch (1:4)	1.39331	0.29986	4.647	3.38e-06 ***
Sex (Male) * Age (5d) * Patch (1:4)	1.11748	0.26220	4.262	2.03e-05 ***
Sex (Male) * Age (25d) * Patch (no patch)	1.63735	0.22446	7.295	3.00e-13 ***
Sex (Male) * Age (5d) * Patch (no patch)	0.95372	0.18926	5.039	4.68e-07 ***
Sex (Male) * Age (25d) * Patch (4:1)	0.64145	0.25708	2.495	0.012592 *
Sex (Male) * Age (5d) * Patch (4:1)	1.12765	0.22613	4.987	6.14e-07 ***

Table S8

Table S8: Full model output for oviposition site selection by mated females at 5, 15 and 25d. Eggs laid on each patch ("successes") Shown here is the summary of the betabinomial generalised linear mixed effects model for all fixed effects. To control for overdispersion and zeroinflation caused by declining fertility with age, a two-column response matrix was constructed by treating eggs paid on each patch in a replicate as "successes" and eggs laid on all other patches as "failures". This response matrix was used as the response variable. Random effects were included for observation (an observation-level random effect for each patch) and replicate.

Predictor	**Estimate**	**Std. error**	**Z value**	**Pr(>|z|)**
(Intercept)	1.71328	0.19507	8.782	1.59e-18 ***
Age (25d)	-1.41810	0.25998	-5.454	4.91e-08 ***
Age (5d)	-0.07728	0.26402	-0.292	0.769744
Patch (1:4)	-3.87531	0.30165	-12.846	8.95e-38 ***
Patch (4.1)	-4.92688	0.36387	-13.539	9.08e-42 ***
Age (25d) * Patch (1.4)	3.01899	0.38018	7.940	2.01e-15 ***
Age (5d) * Patch (1.4)	-0.08327	0.38679	-0.215	0.829548
Age (25d) * Patch (4.1)	1.73508	0.43052	4.030	5.57e-05 ***
Age (5d) * Patch (4.1)	0.63836	0.42407	1.505	0.132251

Table S9

Table S9: Full model output for diet choice in larvae of 5, 15 and 25d parents (both parent sexes age-matched). Shown here is the summary of the Poisson generalised linear mixed effects model for all fixed effects. Random effects were included for replicate and observation (an observation-level random effect for each patch).

Predictor	**Estimate**	**Std. error**	**Z value**	**Pr(>|z|)**
(Intercept)	1.6856	0.1206	13.980	<2e-16 ***
Patch (1:4)	-1.4117	0.2567	-5.499	3.83e-08 ***
Patch (4:1)	-0.8516	0.2109	-4.038	5.39e-05 ***
Parental age (25d)	-0.3123	0.1805	-1.730	0.08356 .
Parental age (5d)	-0.3823	0.1838	-2.080	0.03748 *
Patch (1:4) * Parental age (25d)	0.3606	0.3659	0.985	0.32441
Patch (4:1) * Parental age (25d)	0.6277	0.2927	2.144	0.03201 *
Patch (1:4) * Parental age (5d)	0.9133	0.3419	2.671	0.00756 **
Patch (4:1) * Parental age (5d)	0.4897	0.3028	1.617	0.10581

Table S10

Table S10: Full model output from Cox proportional hazards model for egg-to-adult development time in larvae of 5 and 15d parents reared on 1:4, 1:1 or 4:1 P:C diets. Shown here is the summary of fixed effects from the Cox proportional hazards model, which included fixed effects for parental age and diet and random effects for vial (5 individuals per vial) and individual ID.

Predictor	**coef**	**exp(coef)**	**se(coef)**	**z**	**p**
Parental age	-0.27776	0.75748	0.02878	-9.65	<2e-16
Diet (1:4)	-1.81920	0.16215	0.43086	-4.22	2.42e-05
Diet (4:1)	-0.68032	0.50645	0.45005	-1.51	0.13062
Parental age * Diet (1:4)	0.14908	1.16076	0.03949	3.77	0.00016
Parental age * Diet (4:1)	-0.07998	0.92314	0.04695	-1.70	0.08846

Table S11

Table S11: Full model output from the Conway-Maxwell Poisson generalised linear mixed effects model for egg-to-adult development time in larvae of 5 and 15d parents reared on 1:4 or 1:1 P:C diets; the 4:1 diet had to be excluded from this analysis due to convergence problems which arose from high preadult mortality in larvae of 15d parents reared on this diet. Shown here is the summary of fixed effects from the Conway-Maxwell Poisson model, which included fixed effects for parental age and diet and random effects for vial and individual ID.

Predictor	**Estimate**	**Std. error**	**Z value**	**Pr(>|z|)**
(Intercept)	2.54844	0.01555	163.89	<2e-16 ***
Parental age (5d)	-0.27460	0.02062	-13.31	<2e-16 ***
Diet (1:4)	0.03921	0.02051	1.91	0.0559 .
Parental age (5d) * Diet (1:4)	0.05170	0.02758	1.87	0.0608 .

Table S12

Table S12: Full model output from the binomial generalised linear mixed effects model for preadult mortality (event history analysis) in larvae of 5 and 15d parents reared on 1:4, 1:1 or 4:1 P:C diets. Shown here is the summary of fixed effects. A random effect was included for vial.

Predictor	**Estimate**	**Std. error**	**Z value**	**Pr(>|z|)**
(Intercept)	1.677082	0.278507	6.022	1.73e-09 ***
Parental age (15d)	-1.983303	0.368951	-5.376	7.64e-08 ***
Diet (1:4)	-0.502359	0.369950	-1.358	0.174492
Diet (4:1)	-0.007976	0.386525	-0.021	0.983538
Parental age (15d) * Diet (1:4)	1.487628	0.502203	2.962	0.003054 **
Parental age (15d) * Diet (4:1)	-2.204381	0.569394	-3.871	0.000108 ***

Table S13

Table S13: Full model output from the gaussian generalised linear mixed effects model for thorax length in adult offspring of 5 and 15d old parents. Offspring were reared on 1:4, 1:1 and 4:1 P:C diets. Shown here is the summary of fixed effects. Random effects were included for vial and an observation-level random effect was included for each individual.

Predictor	**Estimate**	**Std. error**	**Z value**	**Pr(>|z|)**
(Intercept)	0.958997	0.013992	68.54	<2e-16 ***
Diet (1:4)	-0.003934	0.018060	-0.22	0.82756
Diet (4:1)	-0.007756	0.023110	-0.34	0.73717
Parental age (5d)	0.022072	0.016890	1.31	0.19128
Sex (Male)	-0.182312	0.008187	-22.27	<2e-16 ***
Diet (1:4) * Parental age (5d)	-0.064289	0.023427	-2.74	0.00607 **
Diet (4:1) * Parental age (5d)	-0.038506	0.027403	-1.41	0.15997

Table S14

Table S14: Full model output from the gaussian generalised linear mixed effects model for wing length in adult offspring of 5 and 15d old parents. Offspring were reared on 1:4, 1:1 and 4:1 P:C diets. Shown here is the summary of fixed effects. Random effects were included for vial and an observation-level random effect was included for each individual. Nonsignificant interactions were retained in this case as they slightly improved model fit (AIC).

Predictor	**Estimate**	**Std. error**	**Z value**	**Pr(>|z|)**
(Intercept)	2.021351	0.026405	76.55	<2e-16 ***
Diet (1:4)	0.132780	0.035177	3.77	0.00016 ***
Diet (4:1)	0.110637	0.046099	2.40	0.01640 *
Parental age (5d)	0.228220	0.033422	6.83	8.58e-12 ***
Sex (Male)	-0.252898	0.034855	-7.26	4.00e-13 ***
Diet (1:4) * Parental age (5d)	-0.370404	0.045887	-8.07	6.91e-16 ***
Diet (4:1) * Parental age (5d)	-0.315623	0.054233	-5.82	5.89e-09 ***
Diet (1:4) * Sex (Male)	-0.005141	0.045767	-0.11	0.91056
Diet (4:1) * Sex (Male)	0.030846	0.058461	0.53	0.59776
Parental age (5d) * Sex (Male)	-0.061697	0.042454	-1.45	0.14616
Diet (1:4) * Parental age (5d) * Sex (Male)	0.086952	0.057944	1.50	0.13345
Diet (4:1) * Parental age (5d) * Sex (Male)	0.022434	0.067919	0.33	0.74117

Table S15

Table S15: Full model output from the gaussian generalised linear mixed effects model for wing length divided by thorax width in adult offspring of 5 and 15d parents. Offspring were reared on 1:4, 1:1 and 4:1 P:C diets. Shown here is the summary of fixed effects. Random effects were included for vial and an observation-level random effect was included for each individual.

Predictor	**Estimate**	**Std. error**	**Z value**	**Pr(>|z|)**
(Intercept)	2.16526	0.05220	41.48	< 2e-16 ***
Sex (Male)	0.12065	0.04958	2.43	0.01495 *
Parental age (5d)	0.10215	0.06604	1.55	0.12192
Diet (1:4)	0.16937	0.06171	2.74	0.00605 **
Diet (4:1)	0.16250	0.07943	2.05	0.04076 *
Sex (Male) * Parental age (5d)	0.11884	0.06116	1.94	0.05200 .
Parental age (5d) * Diet (1:4)	-0.22297	0.07970	-2.80	0.00515 **
Parental age (5d) * Diet (4:1)	-0.24907	0.09356	-2.66	0.00777 **

3.4 Full outputs of emmeans Tukey tests

Table S16

Table S16: Post-hoc Tukey tests comparing how different age groups of mated males and females interacted with diet choice assays.

	Contrast	Estimate	SE	Z ratio	p value
In males					
Landing on 1:4					
	25d - 5d	-0.21	0.14	-1.51	0.29
	15d – 5d	0.04	0.13	0.31	0.29
	15 d - 25d	0.25	0.14	1.83	0.16
Landing on 1:1					
	25d - 5d	-0.95	0.16	-5.91	<0.0001
	15d - 5d	0.34	0.12	2.86	0.01
	15d - 25d	1.29	0.15	8.17	<0.0001
Landing on 4:1					
	25d - 5d	-0.38	0.12	-3.26	<0.01
	15d - 5d	0.02	0.10	0.18	0.98
	15d - 25d	0.40	0.11	3.46	<0.01
No patch					
	25d - 5d	0.46	0.07	6.30	<0.0001
	15d - 5d	-0.18	0.09	-1.99	0.11
	15d - 25d	-0.64	0.08	-7.63	<0.0001
In females					
Landing on 1:4					
	25d - 5d	-0.25	0.18	-1.40	0.34
	15d - 5d	0.35	0.15	2.32	0.05
	15d - 25d	0.60	0.16	3.65	<0.001
Landing on 1:1					
	25d - 5d	-0.67	0.12	-5.61	<0.0001
	15d - 5d	-0.44	0.11	-3.97	<0.001
	15d - 25d	0.23	0.13	1.76	0.18
Landing on 4:1					
	25d - 5d	0.40	0.11	3.49	0.001
	15d - 5d	0.39	0.11	3.39	0.002

	15d - 25d	-0.01	0.10	-0.10	0.99
No patch					
	25d - 5d	0.06	0.04	1.3	0.39
	15d - 5d	-0.02	0.05	-0.48	0.88
	15d - 25d	-0.089	0.05	-1.80	0.17

Table S17

Table S17: Post-hoc Tukey tests comparing the proportion of eggs laid on each diet choice patch by mated females at 5, 15 and 25d post-eclosion.

	Contrast	Estimate	SE	df	Z ratio	p value
Eggs laid on 1:4						
	15d - 25d	-1.60	0.27	Inf	-5.97	<0.0001
	15d - 5d	0.16	0.28	Inf	0.58	0.82
	25d - 5d	1.76	0.26	Inf	6.27	<0.0001
Eggs laid on 1:1						
	15d - 25d	1.41	0.26	Inf	5.45	<0.0001
	15d - 5d	0.08	2.72	Inf	0.29	0.95
	25d - 5d	-1.33	0.26	Inf	-5.31	<0.0001
Eggs laid on 4:1						
	15d - 25d	-0.32	0.33	Inf	-0.97	0.60
	15d - 5d	-0.56	0.32	Inf	-1.77	0.18
	25d - 5d	-0.24	0.30	Inf	-0.82	0.69

Table S18

Table S18: Post-hoc Tukey tests comparing numbers of larvae recovered from each diet patch by parental age group.

	Contrast	Estimate	SE	Z ratio	p value
5d parents					
	1:1 - 1:4	0.50	0.23	2.21	0.07
	1:1 - 4:1	0.36	0.22	1.67	0.22
	1:4 - 4:1	-0.14	0.24	-0.56	0.84
15d parents					
	1:1 - 1:4	1.41	0.26	5.50	<0.0001
	1:1 - 4:1	0.85	0.21	4.04	<0.001
	1:4 - 4:1	-0.56	0.29	-1.95	0.12
25d parents					
	1:1 - 1:4	1.05	0.26	4.03	<0.001
	1:1 - 4:1	0.22	0.20	1.10	0.52
	1:4 - 4:1	-0.83	0.27	-3.08	<0.001

Table S19

Table S19: Post-hoc Tukey tests comparing the probability of eclosion by 14d in larvae reared on the three P:C diet types, separated by parental age group.

	Contrast	Estimate	SE	Z ratio	p value
5d parents					
	1:1 - 1:4	0.50	0.37	1.36	0.36
	1:1 - 4:1	0.01	0.39	0.02	1.0
	1:4 - 4:1	-0.49	0.37	-1.33	0.38
15d parents					
	1:1 - 1:4	-0.99	0.339	-2.90	0.01
	1:1 - 4:1	2.21	0.42	5.28	<0.0001
	1:4 - 4:1	3.20	0.43	7.43	<0.0001

Table S20

Table S20: Post-hoc Tukey tests comparing the probability of eclosion by 14d in larvae of 5d versus 15d parents, separated by rearing diet.

	Contrast	Estimate	SE	Z ratio	p value
1:4 reared					
	15d parents - 5d parents	-0.50	0.34	-1.45	0.15
1:1 reared					
	15d parents - 5d parents	-1.98	0.37	-5.38	<0.0001
4:1 reared					
	15d parents - 5d parents	-4.19	0.46	-9.07	<0.0001

Table S21

Table S21: Post-hoc Tukey tests comparing time to eclosion in days in larvae of 5d versus 15d parents, separated by rearing diet.

	Contrast	Estimate	SE	Z ratio	p value
1:4 reared					
	15d parents - 5d parents	0.22	0.02	12.2	<0.0001
1:1 reared					
	15d parents - 5d parents	0.27	0.02	13.3	<0.0001

Table S22

Table 22: Post-hoc Tukey tests comparing time to eclosion in days in larvae reared on 1:4 or 1:1 P:C diets, separated by parental age group.

	Contrast	Estimate	SE	Z ratio	p value
5d parents					
	1:1 - 1:4	-0.09	0.02	-4.80	<0.0001
15d parents					
	1:1 - 1:4	-0.04	0.02	-1.91	0.06

Table S23

Table S23: Post-hoc Tukey tests comparing wing lengths of male and female offspring with 5d or 15d parents, grouped by rearing diet.

	Contrast	Estimate	SE	Z ratio	p value
Wing length, males					
1:4					
	15d parents - 5d parents	0.12	0.03	3.80	<0.001
1:1					
	15d parents – 5d parents	-0.17	0.03	-5.22	<0.0001
4:1					
	15d parents - 5d parents	0.13	0.04	3.23	0.001
Wing length, females					
1:4					
	15d parents - 5d parents	0.14	0.03	4.20	<0.0001
1:1					
	15d parents - 5d parents	-0.23	0.03	-6.64	<0.0001
4:1					
	15d parents - 5d parents	0.08	0.04	1.75	0.08

Table S24

Table S24: Post-hoc Tukey tests comparing thorax lengths of male and female offspring with 5d or 15d parents, grouped by rearing diet.

	Contrast	Estimate	SE	Z ratio	p value
Thorax length, males					
1:4					
	15d parents - 5d parents	0.06	0.02	2.81	<0.01
1:1					
	15d parents - 5d parents	-0.002	0.02	-0.10	0.92
4:1					
	15d parents - 5d parents	0.05	0.03	1.92	0.06
Thorax length, females					
1:4					
	15d parents - 5d parents	0.02	0.02	1.18	0.24
1:1					
	15d parents - 5d parents	-0.04	0.02	-2.01	0.04
4:1					
	15d parents - 5d parents	-0.03	0.03	-0.98	0.33

Table S25

Table S25: Post-hoc Tukey tests comparing wing length divided by thorax length in male and female offspring with 5d or 15d parents, grouped by rearing diet.

	Contrast	Estimate	SE	Z ratio	p value
Wing/ thorax, males					
1:4					
	15d parents - 5d parents	0.005	0.08	0.06	0.95
1:1					
	15d parents - 5d parents	-0.19	0.09	-2.24	0.03
4:1					
	15d parents - 5d parents	-0.001	0.11	-0.01	0.99
Wing/ thorax, females					
1:4					
	15d parents - 5d parents	0.11	0.06	1.86	0.06
1:1					
	15d parents - 5d parents	-0.15	0.06	-2.33	0.02
4:1					
	15d parents - 5d parents	0.18	0.08	2.24	0.03