Experimental Gerontology 110 (2018) 298–308

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Crowding of *Drosophila* larvae affects lifespan and other life-history traits via reduced availability of dietary yeast

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ARTICLE INFO

Section editor: Sean Curran

Keywords:
Larval crowding
Diet
Lifespan
Fat reserves
Drosophila

ABSTRACT

Conditions experienced during development have often long-lasting effects persisting into adulthood. In *Drosophila*, it is well-documented that larval crowding influences fitness-related traits such as body size, starvation resistance and lifespan. However, the underlying mechanism of this phenomenon is not well understood. Here, we show that the effects of increased larval density on life-history traits can be explained by decreased yeast availability in the diet during development. Yeast-poor larval diet alters various life-history traits and mimics the effects of larval crowding. In particular, reduced amount of yeast in larval diet prolongs developmental time, reduces body size, increases body fat content and starvation resistance, and prolongs *Drosophila* lifespan. Conversely, the effects of larval crowding can be rescued by increasing the concentration of the dietary yeast in the diet during development. Altogether, our results show that the well-known effects of larval crowding on life-history traits are mainly caused by the reduced availability of dietary yeasts due to increased larval competition.

1. Introduction

Developmental conditions are often crucial determinants of adult life-history traits and individual fitness (e.g. Nylin and Gotthard, 1998; Dufty et al., 2002; West-Eberhard, 2003). In natural environments, development under crowded conditions is a frequently occurring phenomenon (e.g. Atkinson, 1979; Griffiths, 1997), which might also represent an important selection agent (Bakker, 1961; Roper et al., 1996). In *Drosophila*, it is well-established that increased larval density has significant impact on various life-history traits. For instance, larval crowding increases developmental mortality (e.g. Sang, 1949; Chiang and Hodson, 1950; Lewontin, 1955; Sokoloff, 1955; Ohba, 1961; Lints, 1963; Miller, 1964; Barker and Podger, 1970; Scheiring et al., 1984; Prout and McChesney, 1985), prolongs developmental time (e.g. Sang, 1949; Sokoloff, 1955; Miller, 1964; Barker and Podger, 1970; Lints and Lints, 1971; Scheiring et al., 1984), reduces body size (e.g. Sang, 1949; Chiang and Hodson, 1950; Sokoloff, 1955; Miller and Thomas, 1958; Miller, 1964; Barker and Podger, 1970; Scheiring et al., 1984; Klepsatel et al., 2014), increases amount of fat reserves (e.g. Zwaan et al., 1991; Baldal et al., 2005), and starvation resistance (e.g. Zwaan et al., 1991; Baldal et al., 2005), decreases fecundity (e.g. Chiang and Hodson, 1950; Lints and Lints, 1971; Prout and McChesney, 1985), alters thermal tolerance (e.g. Quintana and Prevosti, 1990; Loeschcke et al., 1994; Bubli et al., 1998; Sørensen and Loeschcke, 2001; Arias et al., 2012; Henry et al., 2018), and prolongs lifespan (e.g. Miller and Thomas, 1958; Barker and Podger, 1970; Lints and Lints, 1971; Zwaan et al., 1991; Sørensen and Loeschcke, 2001). Comparable effects of high larval density have also been documented in other species (e.g. Park, 1938; Sullivan and Sokal, 1963; Credland et al., 1986; Banks and Thompson, 1987; Ireland and Turner, 2006). Several hypotheses tried to explain how larval crowding affects adult traits, assuming the limited food availability due to scramble competition (e.g. Chiang and Hodson, 1950; Bakker, 1961; Scheiring et al., 1984), increased concentrations of waste products (Botella et al., 1985), scarcity of space (Scheiring et al., 1984), or a combination of all these factors (Henry et al., 2018) to be the main cause. For example, Botella et al. (1985) and Henry et al. (2018) detected an increased concentration of waste products resulting from nitrogen metabolism, i.e. uric acid and urea, in the medium with increased larval density. At high concentrations, these substances have a negative effect on development and survival (Botella et al., 1985). Interestingly, Henry et al. (2018) showed that a small amount of urea in larval food can promote larval cold tolerance but decreases heat resistance. These authors also revealed that larval crowding affects the metabolome profile. Larvae reared at higher densities have increased amounts of sugars, polyols (e.g. arabitol, galactitol) and some acids (e.g. pipecolic acid, fumaric acid), and lower amounts of amino acids

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https://doi.org/10.1016/j.exger.2018.06.016
Received 27 April 2018; Received in revised form 5 June 2018; Accepted 15 June 2018
Available online 20 June 2018
0531-5565/

(Henry et al., 2018).

Larval crowding might represent an environmental stressor that induces hardening effect and generates cross-tolerance to various stresses (e.g. Sørensen and Loeschcke, 2001; Henry et al., 2018). Increased larval density might cause stress-response hormesis with a potentially beneficial effect on longevity (Gems and Partridge, 2008). Numerous studies have shown that larvae kept under high density are more tolerant to heat or cold (Quintana and Prevosti, 1990; Bubli et al., 1998; Sørensen and Loeschcke, 2001; Arias et al., 2012; Henry et al., 2018), while other studies reported no or even decreased heat resistance (Loeschcke et al., 1994; P.K. and M.G., unpublished data). These inconsistencies might reflect differences in experimental methodology (Henry et al., 2018).

Environmental stressors induce a universal cellular response, which is characterized by the production of heat-shock proteins (HSPs) (Feder and Hofmann, 1999). HSPs are involved in protein folding, assembly or degradation of misfolded or damaged proteins (Sørensen et al., 2003). Upregulation of HSPs has been implicated in promoting longevity (reviewed in Tower, 2011). Sørensen and Loeschcke (2001) documented that larvae (but not adults) kept at high density have increased expression of *Hsp70*, one of the major HSPs. However, Henry et al. (2018) did not find any evidence of elevated expression of either HSPs or previously reported antioxidant genes (*catalase*, *superoxide dismutase 1*) (Dudas and Arking, 1995) in larvae kept at increased density. Thus, it seems that altered expression of HSPs is not the major factor responsible for altered thermal tolerance and longevity of flies arising from larvae kept under high density.

Zwaan et al. (1991) presumes that increased lifespan and higher starvation resistance observed in flies that developed under crowded conditions might be a consequence of viability selection. Larval crowding imposes a strong competition for limited resources and thus only the fittest larvae survive to adults. As a consequence, those individuals that manage to survive are on average fitter, which is manifested in their increased longevity and resistance to stressors, such as starvation (Zwaan et al., 1991).

Altogether, several hypotheses on the effects of larval crowding on life-history traits have been proposed, but only few have been experimentally tested (e.g. Botella et al., 1985; Zwaan et al., 1991; Henry et al., 2018). To the best of our knowledge, so far none of them explained phenomena such as increased fat content or prolonged lifespan of flies that developed under high larval density. In this study, we experimentally tested the previously suggested hypothesis that the impacts of larval crowding on life-history traits are caused by food limitation (e.g. Chiang and Hodson, 1950; Bakker, 1961; Scheiring et al., 1984). First, we examined the amount of triglycerides (fat) and proteins in adults that developed at standard (medium) versus high larval densities and that eclosed at different time points. Next, we presumed that changes in protein and fat content might be caused by a depletion of proteins and carbohydrates from a medium due to larval crowding. In that case, these effects could be cancelled out by increasing either carbohydrates or proteins (yeast) in the medium. Previous studies have shown that increase in the dietary sugar prolongs development, reduces size, and increases fat reserves (Musselman et al., 2011; Na et al., 2013). Since these effects resemble the effects of high larval density instead of compensating them, we suspected that changes in physiology and life-history traits of the flies arisen from larvae being kept a high density might be caused by reduced protein availability in the larval medium. We examined here in more detail the hypothesis that the effects of larval crowding on life-history traits are caused predominantly by reduced availability of dietary yeasts (Sang, 1949; Zwaan et al., 1991), which are a natural food for *D. melanogaster* (Begon, 1982) and constitute principal source of proteins (Skorupa et al., 2008) and sterols (Bos et al., 1976). To rigorously test this hypothesis, we manipulated both the larval density and the concentration of yeast in the diet during development and measured the impact of both parameters on numerous traits, such as fat content, starvation resistance, developmental time, body size, and longevity. We show that the reduction of dietary yeast mimics the consequences of larval crowding, whereas increase in the dietary yeast rescued (at least partially) the effect of larval crowding. This proves that larval crowding regulates developmental and life history traits via diminishing the relative amount of dietary yeast per animal.

2. Material and methods

2.1. Fly maintenance and experimental set up

We used a wild-type strain (Canton S obtained from Dr. Halyna Shcherbata, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany) of *D. melanogaster*. Flies were raised at 25 °C (12 h:12 h L:D, 60% humidity) at two egg/larval densities: standard density (150 eggs per 68 ml vial) (Klepsatel et al., 2016) and high density (900 eggs per 68 ml vial). Eggs were collected by allowing 5–10 days old flies (approx. 100 individuals) to lay eggs directly into vials during a three hour period. Eggs were counted using a stereo microscope and any excessive eggs were removed. In addition to standard/normal medium (N) (5.43 g agar, 15.65 g yeast, 8.7 g soy flour, 69.57 g maize flour, 19.13 g blackstrap molasses, 54 g malt, 5.43 ml propionic acid and 1.3 g methyl 4-hydroxybenzoate per 1 l of medium), we used also three additional diets that differed only in the yeast concentration: 6Y (93.9 g yeast), 1/6Y (2.61 g yeast) and 1/12Y (1.3 g yeast). Unless stated otherwise, cohorts of unmated (virgin) males and females that were collected (2–3 times) over a 12 h period (i.e. the maximum age difference between the youngest and oldest individual was 12 h) were kept separately (approx. 30–40 individuals per vial (25 × 95 mm); three to five vials per sex and experimental treatment) on the standard medium and transferred every other day to fresh vials. All experiments were performed at 25 °C (12 h:12 h L:D, 60% humidity).

2.2. Lipid and protein determination

Homogenates of adult flies for lipid and protein measurements were prepared according to Gáliková et al. (2017). Lipid content was determined by a coupled colorimetric assay as described by Gáliková et al. (2015) using the Triglycerides (liquid) assay (Randox, TR1697). Protein content was quantified by the Coomassie Protein Assay (Bradford) Kit (Thermo Scientific, 23200) according to the manufacturer's instructions. Lipids and proteins were measured using the same homogenates (five flies per replicate; three to six replicates per sex and experimental treatment).

2.3. Starvation resistance assay

Starvation resistance was measured in two-day and ten-day-old males and females. Approximately 25–30 flies per replicate were used, and four to five replicates were tested per sex and diet/larval density. For the flies that developed at the normal medium with high larval density, we collected the individuals that eclosed approximately in the middle of the eclosion time range, i.e. on the 16th day after egg laying. All flies were placed onto 1% agarose (in water), and the number of dead flies was examined every 6–12 h (at 9 AM, 3 PM, and 9 PM).

2.4. Developmental time

Developmental time was measured as the time from the egg laying to the eclosion. Eggs were collected during a three hour period as described above. The number and sex of eclosed flies was examined every 12 h until the last fly eclosed.

2.5. Wing size measurements

Wing area was examined in two-day old males and females (25