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Gene-environment interplay in *Drosophila melanogaster*: Chronic nutritional deprivation in larval life affects adult fecal output

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ABSTRACT

Life history consequences of stress in early life are varied and known to have lasting impacts on the fitness of an organism. Gene-environment interactions play a large role in how phenotypic differences are mediated by stressful conditions during development. Here we use natural allelic 'rover/sitter' variants of the *foraging* (*for*) gene and chronic early life nutrient deprivation to investigate gene-environment interactions on excretion phenotypes. Excretion assay analysis and a fully factorial nutritional regimen encompassing the larval and adult life cycle of *Drosophila melanogaster* were used to assess the effects of larval and adult nutritional stress on adult excretion phenotypes. Natural allelic variants of *for* exhibited differences in the number of fecal spots when they were nutritionally deprived as larvae and well fed as adults. *for* mediates the excretion response to chronic early-life nutritional stress in mated female, virgin female, and male rovers and sitters. Transgenic manipulations of *for* in a sitter genetic background under larval but not adult food deprivation increases the number of fecal spots. Our study shows that food deprivation early in life affects adult excretion phenotypes and these excretion differences are mediated by *for*.

1. Introduction

The life history of an organism can often be separated into distinct stages, with discrete periods of development, that have the potential to influence later stages. The extent, direction, and permanence of these stages are dependent on a variety of internal and external factors. The environment acts as a major entity of lasting consequence on an organism's life history (Kloss et al., 2009; Marshall and Sinclair, 2010; McDade, 2012; Yang et al., 2012). Environmental factors such as temperature (Bochandanovits and de Jong, 2003; Sorensen and Loeschcke, 2001), social environment (Bagot et al., 2012; Krupp et al., 2008; Levine et al., 2002; Schneider et al., 2012), and nutritional composition (Burns et al., 2012; Fitzpatrick et al., 2007; Scrimshaw, 1998) influence early life experience and affect adult fitness.

Nutritional stress can occur on acute or chronic time scales. Studies investigating acute nutritive stress focus on starvation resistance during short, discrete developmental time periods (Lin et al., 2013). Studies that investigate chronic nutritional stress focus on long-term consequences of nutritional stress within (Kaun et al., 2007a) and between life history stages (Burns et al., 2012), throughout life (Kristensen et al., 2011), as well as in natural selection experiments performed on evolutionary time scales (Kloss et al., 2009; Sokolowski et al., 1997). Nutritional composition of an environment has the potential to vary in uniformity, quality, and complexity. Ratios of nutrients and total quantity of calories available within and between habitats can be experimentally manipulated (Piper et al., 2005; Skorupa et al., 2008). The ability to thrive in a chronically nutrient restricted environment can be evolutionarily advantageous (Vijendravarma et al., 2012a). This suggests that gene-environment interactions contribute to how well negative stressors are buffered. In addition, gene-environment interactions early in life may influence response to adversity later in life (Boyce et al., 2012).

In the current study we use *Drosophila melanogaster*, a genetically tractable model organism, to investigate the effects of nutritional stress. *D. melanogaster* has a complex life cycle with distinct larval and adult stages. Here we investigate the effects of chronic food deprivation in the larval and/or adult periods of development on adult excretion by using the rover and sitter natural allelic variants of the *foraging* (*for*) gene (de Belle et al., 1989; Osborne et al., 1997). The *for* gene was originally discovered through mapping phenotypic variation in larval movement

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patterns on a yeast water paste (Sokolowski, 1980); rover larvae move more while foraging for food than sitter larvae (Sokolowski, 2001). *for* encodes a cGMP-dependent protein kinase G (PKG) which underlies the extensive pleiotropy and plasticity in both larval and adult *D. melanogaster* behavioral and metabolic phenotypes (Burns et al., 2012; Kaun et al., 2007a; review Reaume and Sokolowski, 2009). When adult heads and larval nervous systems are assayed, rovers have higher *for* mRNA expression levels and PKG enzyme activity levels than sitters (Kaun et al., 2007a; Osborne et al., 1997). A previous study showed that chronic larval but not adult food deprivation interacts with the *for* gene to affect darting-exploration defined as the extent to which adult flies move from the edge of an open field and dart into the center (Burns et al., 2012). The above studies suggest that *for* interacts with the nutritional context to affect a number of phenotypes.

In the current study, we investigate the adult excreta of rovers and sitters under varying nutritional regimes in the larval and adult life history stages using a protocol adapted from Cognigni et al. (2011). Quantifying excretion phenotypes allows for a visual readout of visceral processes that are associated with nutritional need, regulation, and function (Cognigni et al., 2011). Excretion analysis can act as a proxy for gut function providing information about a highly nutrient sensitive tissue (O'Brien et al., 2011) and a hub for whole-animal nutrient homeostasis (Cognigni et al., 2011). PKG has been associated with many phenotypes arising from function both inside (Burns et al., 2012; Donlea et al., 2012; Kaun et al., 2007b; Kuntz et al., 2012; Mery et al., 2007; Wang et al., 2008) and outside the brain (Dow et al., 1994a,b; Klein et al., 2013). However, little is known about the interplay between allelic variation in *for* and early life nutritional adversity on adult excretion phenotypes.

2. Materials and methods

2.1. Fly strains

The homozygous for^R (rover) and for^s (sitter) strains described in Fitzpatrick et al. (2007) were reisogenized in 2013 and their first and third chromosomes were made co-isogenic.

Transgenic expression of *for* was accomplished using the UAS-GAL4 system (Brand and Perrimon, 1993). A *UAS-forT1* line described in Donlea et al. (2012) and Kuntz et al. (2012) were crossed to a *hs-GAL4* line described in Kaun et al., (2007a). The UAS line was backcrossed into a w^{118}; for^s genetic background using 9–10 generations of backcrossing after which the *X*-chromosomes were replaced with wild-type for^s *X*-chromosomes to remove any phenotypic effects of w^{118} alleles. The *hs-GAL4* line was also on a for^s genetic background. We crossed the UAS line to the *hs-GAL4* line and raised the test populations in the nutrient treatment conditions (described below) for the excretion assay at 25 °C as in Kaun et al. (2007a). Controls were crosses of each of the GAL4 and UAS lines with the isogenized for^s strain.

Strains were maintained in 6 oz bottles on a 45 mL standard yeast-sugar-agar medium (1000 mL H_2O, 100 g sucrose, 50 g Fleischmann's yeast, 16 g agar, 8 g $C_4H_4KNaO_6$, 1 g KH_2PO_4, 0.5 g NaCl, 0.5 g $MgCl_2$, 0.5 g $CaCl_2$, 0.5 g $Fe_2(SO_4)_3$) at 25 °C with 40–60% humidity under a 12 h L:D cycle with lights on at 0800 h (standard conditions).

2.2. Nutritional treatments

Standard food medium was designated as high (H) and nutrient deprivation food medium was designated as low (L). Low food consisted of an 85% reduction in both yeast and sugar compared to standard food. Larval and adult flies were exposed to the following combinations of L and H food: HH, LH, HL LL, with the first letter showing the quality of food available throughout larval development and the second letter showing the quality of food available during the adult stage.

2.3. Excretion assay

The protocol for measuring excretion in adult flies was modified from Cognigni et al. (2011). To prepare adult flies for the excretion assay, 20 first instar larvae ±2 h in age were seeded into 50 mL vials containing 10 mL of L or H food medium. Larvae developed in incubators under standard conditions. Flies eclosed between 9 and 14 days post-hatch depending on the nutrient treatment with the LL and LH treatment flies taking longer to eclose. Adult flies were transferred ±5 h post-eclosion (O'Brien et al., 2011) via light CO_2 anesthetic into L or H food medium vials depending on the nutrient treatment they were randomly assigned to. All adult food treatments included 0.5% Bromophenol blue (BPB). Male and female adult flies were housed separately with $n = 20$ flies per vial. Mated female test flies were generated by housing 14 females with 6 males for 3 days and it was unknown at which time point mated status was achieved.

When flies reached 3-days-old, four adult flies (virgin female, mated female or male) were aspirated into each 60mm × 15 mm petri dish. Each dish contained a food puck (1 cm^3, supplemented with 0.5% BPB) made from L or H food according to adult treatment. The sample size for the excretion assays consisted of 30 dishes per sex/condition (virgin female, mated female or male) and per food treatment (HH, LH HL, LL).

For the excretion assay, petri dishes were labeled in a double blind procedure and placed lid side down alternating between empty and treatment dishes in a randomized position in wire cages complete with an empty petri dish border. Dishes remained undisturbed in an incubator at standard conditions for 24 h ± 0.5h. Flies were then discarded and petri dish lids and bottoms were scanned using an HP LaserJet scanner at a high-resolution (1200 × 1200) color setting. Images were saved as Jpeg files and adjusted for color, brightness, contrast, and exposure with Adobe Photoshop. Images were analyzed by counting the total number of fecal spots and visually analyzing differences in the shape of excretion per petri dish (lid and bottom). Differences in excrement shape were visually quantified as large and round (round) or small, thin, and rod shaped (Reproductive Oblong Deposit – ROD) as described in Cognigni et al. (2011).

2.4. CAFÉ assay

We modified the CAFÉ assay from Ja et al. (2007) to quantify the volume (μL) consumed over a 24 h period by mated female rovers, sitters, transgenic flies and controls who experienced all combinations of nutritional environments (HH, LH, HL, LL). Larvae and adults were reared as described above. Three-day-old mated adult females were individually aspirated into the CAFÉ assay and allowed to acclimatize for 24 h prior to quantification of their food intake in the 24 h CAFÉ assay. The CAFÉ assay took place in an environmental chamber at standard conditions with $n = 30$ per treatment. Flies that did not feed in the CAFÉ assay were not included in the analyses. The proportion of flies that did not feed during the CAFÉ assay did not differ between strains and treatments. Flies were housed individually in 50 mL vials with 20 mL water and packed cotton to prevent desiccation. A calibrated glass micropipette (5 μL) filled with liquid food medium by capillary action was inserted into the lid of the test vial. Two different CAFÉ assay liquid food treatments were used to complement the H and L excretion assay conditions. High CAFÉ assay food (CAFÉ H: 100 mL H_2O, 10 g sucrose, 5 g autolyzed yeast) and low CAFÉ assay food (CAFÉ L: 100 mL H_2O, 1.5 g sucrose, 0.75 g autolyzed yeast) were modified to contain 0.5% BPB dye and matched to adult nutrient conditions. Multiple control capillaries were used in each test row to determine evaporative losses of both CAFÉ H and