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Food intake and food choice are altered by the developmental transition at critical weight in *Drosophila melanogaster*

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An animal's metabolism changes throughout development, obliging the animal to coordinate its feeding behaviour with its stage-specific nutritional requirements. Previous studies in the fruit fly *Drosophila melanogaster* showed that the developmental transition known as critical weight alters the response to nutrition in larvae; starvation reduces survival and dramatically delays development in precritical weight larvae, whereas it has more moderate effects on survival and accelerates development in postcritical weight larvae. We thus hypothesized that this change in sensitivity to nutrition might result in differences in feeding behaviour between the two stages. Using both no-choice and two-choice assays, we found that pre- and postcritical weight larvae had similar strategies for macronutrient balancing, both regulating protein intake at the cost of under- or overconsuming carbohydrates. Despite these similarities, precritical weight larvae regulated protein intake within more narrow limits than postcritical weight larvae. In addition, the larvae showed significant differences in the way they regulated macronutrient intake in the presence of bitter, potentially noxious compounds. Whereas precritical weight larvae avoided bitter food and showed only mild deficiencies in protein intake, postcritical weight larvae responded to these compounds by consuming less. When larvae were forced to choose between a higher quality diet tainted with quinine or caffeine and a lower quality diet containing less protein, larvae of both stages showed similar avoidance strategies but precritical weight larvae maintained a more constant protein intake than their postcritical weight siblings. Together, our results show that the developmental transition at critical weight modifies larval feeding behaviour, increasing our understanding of how developmental processes influence behaviour.

The consequences of poor nutrition for animal growth, development and life history change with developmental stage. Early, prolonged exposure to poor nutrition in juvenile stages has the potential to affect more life history traits than in adult stages (Barrett, Hunt, Moore, & Moore, 2009; Dmitriew & Rowe, 2007, 2011; Metcalfe & Monaghan, 2001; Monaghan, 2008; Taborsky, 2006). Further, sensitivity to poor nutrition, including its effects on survival, growth and metabolism, changes over the course of development (Koyama, Mendes, & Mirth, 2013; Lee, Kwon, & Roh, 2012; Simpson & Raubenheimer, 2012). For example, in the caterpillar *Spodoptera litura*, feeding sixth-instar larvae on low-protein diets affects survival more than in the fifth instar (Lee et al., 2012). However, how the changing nutritional requirements of developing animals are reflected in their food intake and food choices has received little attention (Carvalho & Mirth, 2015; Lee et al., 2012).

Animals choose what and how much to eat based on a variety of interacting factors including their nutritional requirements, satiety state, reproductive state, food composition and presence of defensive or toxic chemicals (Carvalho & Mirth, 2015; Lee et al., 2012). Classically, food choice has been explained using optimal foraging models which describe foraging behaviour as a function of a nutritional currency, typically either energy or single limiting nutrients, and foraging costs, including energy lost through foraging or food toxicity (Emlen, 1966; Emlen & Emlen, 1975; Pulliam, 1974; Rapport, 1971; Stephens & Krebs, 1986). These models have contributed crucial insight into how animals make food-related decisions, including whether to exploit, and how long to remain in, a food patch (Emlen, 1966; Krebs, Ryan, & Charnov, 1974; MacArthur & Pianka, 1966; Wolf, Hainsworth, & Gill, 1975). However, they do not account for the fact that animals require

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multiple nutrients for their growth, survival and reproductive success (Raubenheimer, Simpson, & Mayntz, 2009; Simpson & Raubenheimer, 2012).

More recent work has highlighted the importance of interactions between nutrients in the diet in determining what and how much animals eat, particularly in the context of the geometric framework for nutrition (Raubenheimer & Simpson, 1997, 1999; Simpson & Raubenheimer, 1999). This approach creates a nutrient space by varying two nutrients across a broad range, and exploring how the interactions between nutrients affect foraging decisions and the animal's biology. These studies have shown how the balance of nutrients in an animal's diet affect food intake, food choice and a wide range of life history traits in organisms as diverse as slime moulds, humans, cats, dogs, spiders and insects (Dussutour, Latty, Beekman, & Simpson, 2010; Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Hewson-Hughes et al., 2013; Jensen et al., 2012; Lee, Cory, Wilson, Raubenheimer, & Simpson, 2006, 2008; Mayntz, Raubenheimer, Salomon, Toft, & Simpson, 2005; Simpson, Batley, & Raubenheimer, 2003; Simpson & Raubenheimer, 2012). This approach provides an excellent framework for understanding how nutritional requirements and food choice behaviour change with developmental stage.

Insects show several food-related behaviours that change throughout development. In the larvae of the fruit fly *Drosophila melanogaster*, digging activity increases with larval age (Godoy-Herrera, 1986), and larvae change from active foraging to food avoidance during the wandering stage at the end of larval development (Sokolowski, Kent, & Wong, 1984). In addition to this transition to food avoidance at wandering, a developmental transition early in the third (final) larval instar (L3) known as critical weight determines how starvation affects survival and developmental time. Larvae starved before reaching critical weight show low survival and those that do survive significantly prolong the time to pupariation (Beadle, Tatum, & Clancy, 1938; Koyama, Rodrigues, Athanasiadis, Shingleton, & Mirth, 2014; Mirth, Truman, & Riddiford, 2005; Shingleton, Das, Vinicius, & Stern, 2005). When starved after critical weight, larvae show higher rates of survival and pupariate earlier than fed larvae (Beadle et al., 1938; Koyama et al., 2014; Mirth et al., 2005; Stieper, Kupershtok, Driscoll, & Shingleton, 2008). Given its effects on survival and developmental time in response to nutrition, we hypothesized that critical weight also acts to modify feeding behaviour.

Regulating developmental time plays an important role in shaping larval food choices. Our previous work showed that while survival from egg to pupariation and female and male body size were maximized in high-protein, low-carbohydrate diets, larval developmental time was minimized in intermediate protein to carbohydrate ratio diets (Rodrigues et al., 2015). When choosing between two foods of differing protein and carbohydrate concentrations, third-instar larvae regulated their protein and carbohydrate intake towards the dietary conditions that minimized developmental time (Rodrigues et al., 2015). Because critical weight alters how starvation affects development time, these findings provide further support for our hypothesis that critical weight changes food choice behaviour and rates of food intake.

In the wild, *D. melanogaster* larvae utilize rotting fruit and other decaying organic matter. As fruit decays, it becomes colonized by a succession of microorganisms that change its macronutrient content, chemical composition and pH (Lachaise, Tsacas, & Couturier, 1982; Matavelli, Carvalho, Martins, & Mirth, 2015; Morais, Martins, Klaczko, Mendonça-Hagler, & Hagler, 1995; Nunney, 1990). Larvae choose between food patches within the rotting fruit and choose how much food to ingest based on the composition of a particular patch. Changes in the food substrate over time are expected to impact larval food choices, as larvae seek to meet their nutritional requirements and avoid chemicals produced by the microbial community, conspecifics and other animals exploiting the patch (Anagnostou, LeGrand, & Rohlfs, 2010; Beltramí, Medina-Muñoz, Del Pino, Ferveur, & Godoy-Herrera, 2012; Mast, Moraes, Alborn, Lavis, & Stern, 2014). In other insects, such as the locusts *Schistocerca gregaria* and *Locusta migratoria* and the German cockroach, *Blatella germanica*, the presence of aversive compounds in the food alters dietary preferences and nutrient intake (Jensen, Schal, & Silverman, 2015; Raubenheimer & Simpson, 1990; Shik, Schal, & Silverman, 2014; Simpson & Raubenheimer, 2001). Given this, in addition to altering their food choices to meet stage-specific nutritional requirements, we predicted that avoidance responses towards noxious compounds might also change with developmental stage.

In this study, we used the geometric framework for nutrition to explore how critical weight modified the way larvae (1) regulated their food choice and macronutrient intake, (2) changed their food choice to avoid bitter, potentially noxious compounds in the diet and (3) regulated their food choice and macronutrient intake when choosing between low-quality food and higher quality food tainted with noxious chemicals. Given that precritical weight larvae are more sensitive to starvation, we expected that they would show tighter regulation of their macronutrient intake, stronger avoidance of noxious compounds and be less willing to accept poor quality food over higher quality food tainted with noxious chemicals.

METHODS

Fly Stocks and Stock Maintenance Conditions

Drosophila melanogaster white^{1118} (w^{1118}) was obtained from the University of Cambridge. We chose this mutant strain as it is a commonly used control for behavioural screens, thus facilitating comparisons between studies. One caveat with using w^{1118} mutants is that *white* encodes an ABC transporter known to alter the distribution of biogenic amines in the nervous system, sometimes generating behavioural defects (Borycz, Borycz, Kubów, Lloyd, & Meinertzhagen, 2008). Fly stocks were maintained as overlapping generations in 250 ml fly bottles at 25 °C, in a 12:12 h light:dark regime and 60–70% humidity during stock maintenance. Experiments were performed under the same conditions apart from being in complete darkness. Adults were kept on standard food used in the laboratory, which included 45 g/litre molasses, 75 g/litre sucrose, 70 g/litre corn flour, 20 g/litre yeast extract, 10 g/litre agar and 0.25% nipagen.

Experimental Diets

Because *D. melanogaster*'s natural food source contains primarily yeast and sugars, we produced different foods by varying the amount of yeast (Lesaffre SAF-Instant Red no. 15909, 31105, 31150, which contains 50 g protein and 33 g carbohydrate per 100 g) and sucrose (Sidul, Santa Iria de Azóia, Portugal, which contains 100 g carbohydrates per 100 g). We also added 0.5% agar and 0.25% nipagen to each diet, to control consistency and prevent fungal growth, respectively. In addition, we autoclaved the diets before distributing them into tissue culture dishes (55 mm diameter). Diets differed in caloric content, either 0.75 kJ/ml or 1.51 kJ/ml (45 g/litre and 90 g/litre, respectively), and protein to carbohydrate (P:C) ratio, either 1:32, 1:16, 1:8, 1:4, 1:2, 1:1 or 1.5:1.

Larval Staging

To assess behaviour in pre- and postcritical weight larvae, we carefully staged the larvae from the moult to the third larval instar.