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RESEARCH ARTICLE

Drosophila females trade off good nutrition with high-quality oviposition sites when choosing foods

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ABSTRACT

Animals, from insects to humans, select foods to regulate their acquisition of key nutrients in amounts and balances that maximise fitness. In species in which the nutrition of juveniles depends on parents, adults must make challenging foraging decisions that simultaneously address their own nutrient needs as well as those of their progeny. Here, we examined how the fruit fly *Drosophila melanogaster*, a species in which individuals eat and lay eggs in decaying fruits, integrate feeding decisions (individual nutrition) and oviposition decisions (offspring nutrition) when foraging. Using cafeteria assays with artificial diets varying in concentrations and ratios of protein to carbohydrates, we show that *D. melanogaster* females exhibit complex foraging patterns, alternating between laying eggs on high carbohydrate foods and feeding on foods with different nutrient contents depending on their own nutritional state. Although larvae showed faster development on high protein foods, both survival and learning performance were higher on balanced foods. We suggest that the apparent mismatch between the oviposition preference of females for high carbohydrate foods and the high performances of larvae on balanced foods reflects a natural situation where high carbohydrate ripened fruits gradually enrich in proteinaceous yeast as they start rotting, thereby yielding optimal nutrition for the developing larvae. Our findings that animals with rudimentary parental care uncouple feeding and egg-laying decisions in order to balance their own diet and provide a nutritionally optimal environment to their progeny reveal unsuspected levels of complexity in the nutritional ecology of parent–offspring interactions.

KEY WORDS: *Drosophila melanogaster*, Fruit fly, Nutritional geometry, Foraging behaviour, Feeding, Egg-laying

INTRODUCTION

Animals have evolved sophisticated nutritional strategies to locate, select and ingest blends of nutrients maximising growth and reproduction (Simpson and Raubenheimer, 2012). Over the past decades, comparative research in nutritional ecology has showed how individual animals efficiently self-regulate their intake of multiple nutrients simultaneously and how this varies across developmental stages, taxonomic groups and feeding guilds (Behmer, 2009; Simpson and Raubenheimer, 1993, 2012; Simpson et al., 2015a,b). However, much less is known about how these complex regulatory behaviours are affected by social and competitive interactions in groups and populations (Lihoreau et al., 2014, 2015; Senior et al., 2015, 2016; Simpson et al., 2010). Many animals use social information provided by conspecifics to select food resources (Danchin et al., 2004; Giraldeau and Caraco, 2000). Therefore, under these conditions, an individual's decision to eat a food depends not only on its own nutritional requirements, but also on the requirements of others, including social partners and competitors (Lihoreau et al., 2014). These trade-offs between optimising individual nutrition and interacting socially can have important consequences on group-level phenomena, such as social structures and collective dynamics (Lihoreau et al., 2015). For instance, in advanced social insects, such as ants and bees, food collection is achieved by a subset of individuals (the foragers) that must integrate their own nutrient needs as well as the different needs of all other nestmates, including workers, breeders (queens) and the brood (eggs and larvae) when deciding which food to collect (Dussutour and Simpson, 2009). Foragers compensate for specific nutrient deficiencies to maintain a colony-level intake target that varies with colony composition and developmental stage [e.g. ants (Christensen et al., 2010; Cook et al., 2010; Dussutour and Simpson, 2009, 2012), honeybees (Altaye et al., 2010; Hendriksma and Shafir, 2016) and bumblebees (Stabler et al., 2015)].

Although most research on dietary alloregulation (when individuals make nutritional decisions for others) has focused on social insects (Simpson et al., 2015a), in principle, similar strategies could be observed in all parent–offspring associations in which juveniles do not actively forage or do not choose their foraging environment. At the most simplistic level, females must find a suitable breeding site for the development of the juveniles (Royle et al., 2012). In species in which animals lay eggs in food resources, such as fruit flies, the challenge for the females is to trade off between choosing food substrates maximising their own nutrition and providing a high-quality nutritional environment for the development of their offspring (Reaume and Sokolowski, 2006). Because fruit fly larvae have limited mobility, their nutrition is largely determined by the mother's choice of oviposition site, making egg-laying decisions crucial for the survival of embryos and larvae.

Recent studies using nutritional geometry, a conceptual approach to dissect the nutritional interactions between animals and their environment (Simpson and Raubenheimer, 1993, 2012; Simpson et al., 2015b), have shown how fruit flies actively balance their acquisition of macronutrients (protein and carbohydrates) to trade off fitness traits such as development time, reproduction and survival [e.g. *Drosophila melanogaster* (Lee, 2015; Lee et al., 2008, 2013; Piper et al., 2014; Reddiex et al., 2013; Ribeiro and Dickson, 2010; Rodrigues et al., 2015); other fruit flies (Fanson et al., 2009; Matavelli et al., 2015)]. These effects of nutrition on physiology and behaviour greatly vary with age, sex and the mating status of individuals. For instance, when provided with nutritionally

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complementary diets, mated females and larvae balance their intake of protein (P) and carbohydrates (C) to reach P:C ratios maximising growth and reproduction [mated females P:C 1:1.5 (Lee et al., 2008, 2013), larvae P:C 1:2 (Rodrigues et al., 2015)], whereas unmated females and males tend to consume more carbohydrates for energy [P:C 1:4 (Lee et al., 2013)]. Several studies also indicate that *D. melanogaster* females are highly selective when choosing oviposition sites (Yang et al., 2008). Although yeast is an important cue for attracting flies to food resources (Becher et al., 2012), females prefer laying eggs on substrates rich in carbohydrates, such as sucrose-based media (Schwartz et al., 2012) or mixed foods with low P:C ratios (Rodrigues et al., 2015) [but see (Yang et al., 2008)], suggesting that flies choose foods with a suboptimal nutrient balance for the development of their future larvae.

One hypothesis to reconcile these laboratory studies is that *D. melanogaster* females anticipate the gradual change of nutrient content in their natural food resources (decaying fruits) that may occur throughout larval development. Maturation of fruits, from ripening to rotting, is accompanied by important modifications in the density and diversity of yeast populations (Morais et al., 1995), resulting in predictable variations in P:C ratios with the stage of fruit decay (Tournas and Katsoudas, 2005; Matavelli et al., 2015). Alternately, females may simply lay eggs on the foods they eat from. Under this second hypothesis, oviposition choices may be primarily driven by the nutrient needs (nutritional state) of females. The strong preferences for laying eggs in high carbohydrate foods observed in previous studies (Rodrigues et al., 2015; Schwartz et al., 2012) may thus result from an attempt of flies bred on high protein diets to compensate for their deficit in carbohydrates (Lee et al., 2008, 2013).

Here, we explored how *D. melanogaster* flies integrate feeding and oviposition decisions when choosing food resources. First, we used nutritional geometry to test the importance of nutrient balance (P:C) and concentration (P+C) on female foraging behaviour. We measured the oviposition preferences of females in multiple-choice (cafeteria) assays and manipulated the nutritional state of females to test the relative importance of oviposition and feeding in food choices. Next, we examined the consequences of female oviposition choices on the fitness of their progeny by comparing growth, survival and cognitive performances of larvae bred on diets with different nutrient ratios. Cognitive impairments were assessed in an olfactory learning task where larvae had to associate an odour with a food reward.

MATERIALS AND METHODS

Fly culture

Wild-type Canton-S *D. melanogaster* flies (Bloomington *Drosophila* Stock Center) were reared under standard conditions (20°C, 60% relative humidity, 12 h:12 h light:dark photoregime, light on at 08:00 h). Flies were cultured in 150 ml plastic bottles containing standard diet made of dry inactive yeast (90 g l^{-1}, Dutscher Scientific, Brentwood, UK), maize flour (90 g l^{-1}, Genesee Scientific, San Diego, CA, USA), Vanderzant vitamin mixture for insects (0.25 g l^{-1}, Sigma-Aldrich, St Louis, MO, USA), Tegosept (4 g l^{-1}, Dutscher Scientific) and propionic acid (1.5 g l^{-1}, Dutscher Scientific) in a 1.5% agar gel (Dutscher Scientific). The protein to carbohydrate (P:C) ratio of the standard diet was 1:2.

Experiments 1–7 were conducted with 4-day-old mated females. To obtain mated females, virgin adults were collected from the stock culture within 2 h of eclosion from the pupae and maintained in groups of 15 males and 15 females in culture bottles with standard diet (experiments 1–3) or experimental diet (experiments 4–7) for mating. After 96 h, females were transferred to a test arena (experiments 1–5) or to plastic tubes (experiments 6–7) under light CO_2 anaesthesia (see details below). All experiments were conducted in climate-controlled chambers (20°C, 60% relative humidity) under far-red light (LED bulb 625–630 nm, Rubin-Lacaque), which is not detected by flies (Heisenberg and Buchner, 1977). All experiments were started at 10:00 h. For cafeteria assays (experiments 2–5), the different diets were placed in a circular array and their relative positions were pseudo-randomised at each trial to avoid potential biases due to side preferences or hard-wired foraging movement rules by flies.

Experimental diets

We designed 34 experimental diets differing in their content of protein and digestible carbohydrates. The protein content was manipulated using a mix of whey protein and casein (ratio whey: casein 1:4, Nutrimuscle, Longwy, France). The carbohydrate content was manipulated using sucrose (Dutscher Scientific). The quantity of yeast (dry and inactive, Dutscher Scientific) was kept constant (10 g l^{-1}) in order to keep the quantity of minerals and other components present in yeast identical across all diets. The protein and carbohydrate contents of the yeast (0.45 g g^{-1} protein, 0.24 g g^{-1} carbohydrate) were included in the calculation of the protein to carbohydrate ratios tested. Vanderzant vitamin mixture for insects (2.5 g l^{-1}, Sigma-Aldrich), Tegosept (4 g l^{-1}, Dutscher Scientific) and propionic acid (1.5 g l^{-1}, Dutscher Scientific) were added to each diet. All diets were presented to the insects in a 2% agar gel (Dutscher Scientific), providing suitable feeding and oviposition sites.

Experiment 1: egg-laying performances

We assessed the egg-laying performances of females reared on standard diet, confined to one of 34 experimental diets varying in protein and carbohydrate content, using four nutrient concentrations (P+C 45, 90, 180 and 270 g l^{-1}) and 10 nutrient ratios (P:C 1:56, 1:32, 1:16, 1:8, 1:4, 1:2, 1:1, 2:1, 4:1 and 8:1). Each fly was tested for 24 h in a small Petri dish (Ø=35 mm, height=15 mm) filled with 5 ml of diet. At the end of the test, the fly was removed and the number of eggs laid on the food was counted. The experiment was repeated at least 20 times for each diet (N=808 flies; see details in Table S1).

Experiment 2: egg-laying preferences

We assessed the egg-laying preferences of females reared on standard diet in a cafeteria assay. Flies were tested for 24 h, during which they had unrestricted access to eight patches of different experimental diets (Ø=35 mm, height=15 mm, volume=5 ml) set in a 15 ml agar gel basis (30 g l^{-1}) in a large Petri dish (Ø=145 mm, height=20 mm). Diets varied in their nutrient ratios (P:C 1:16, 1:8, 1:4, 1:2, 1:1, 2:1, 4:1 and 8:1, P+C 180 g l^{-1}; Table S1). At the end of the test, flies were removed and the number of eggs laid on each diet was counted. Flies were tested either alone (N=40 flies) or in groups of 10 (N=24 groups; Table S1).

Experiment 3: interaction between feeding and egg-laying preferences

We examined the feeding and egg-laying preferences of flies reared on standard diet in cafeteria assays with eight patches of different experimental diets, similar to experiment 2. The flies were observed for 24 h. Top-view pictures of the test arena were taken every minute with a webcam (HD Webcam C270, Logitech, Romanel-sur-

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Morges, Switzerland) placed 150 mm above the setup and programmed with Zone Trigger (Omega Unfold, Montreal, QC, Canada). The number of flies on each food patch was counted on each of 80,640 images recorded using the 'analyse particles' tool in ImageJ (National Institutes of Health, Bethesda, MD, USA; for details of the image analysis procedure, see Lihoreau et al., 2016). At the end of the test, flies were removed and the number of eggs laid on each patch was counted. The experiment was repeated 21 times (N=21 groups of 10; Table S1).

Assuming that flies were eating when they were on a food patch, we estimated the cumulated intake of protein (I_P) and carbohydrate (I_C) by flies based on time spent on food:

$$I_P = \sum_{t=1}^{x} \sum_{i=1}^{8} \frac{T_i \times P_i}{N}, \quad (1)$$

$$I_C = \sum_{t=1}^{x} \sum_{i=1}^{8} \frac{T_i \times C_i}{N}, \quad (2)$$

where t is the time since the beginning of the experiment (0 to 1440 min), N is the number of flies in the cafeteria, T_i is the cumulated time spent on food patch i, and P_i and C_i are the concentrations in protein and carbohydrate in food patch i, respectively. For simplicity, we assumed that time spent on food correlates with food consumption and considered that flies ate from each diet at the same constant rate (for finer-scale patterns, see Itskov et al., 2014). We did not consider the time spent laying eggs on food, which is typically accomplished within 1 min (Yang et al., 2008) and is therefore negligible for the duration of our observations.

Experiment 4: effect of nutritional state on egg-laying performances and preferences

We examined the egg-laying preferences of flies maintained on different breeding diets varying in nutrient concentrations and ratios. Flies were transferred to a high carbohydrate diet (P:C 1:16, P+C 180 g l^{-1}), a high protein diet (P:C 8:1, P+C 180 g l^{-1}) or an intermediate diet (P:C 1:2, P+C 180 g l^{-1}) within 2 h of emergence from the pupae, and maintained under these conditions for 96 h. We used a first batch of flies to investigate the role of nutritional state in egg production. Flies were tested individually in a no-choice assay similar to experiment 1 but with standard diet (N=40 flies per nutritional state; Table S1). We used a second batch of flies to investigate the role of nutritional state in egg-laying preferences. Flies were tested in groups of 10 in one of four cafeteria assays containing eight patches of experimental diets with different P+C concentrations: 45, 90, 180 and 360 g l^{-1}. The following cafeterias were used: P:C 1:8, 1:6, 1:4, 1:2, 1:1, 2:1, 4:1 and 8:1 at P+C 45 g l^{-1}; P:C 1:16, 1:8, 1:4, 1:2, 1:1, 2:1, 4:1 and 8:1 at P+C 90 g l^{-1}; P:C 1:32, 1:16, 1:8, 1:4, 1:2, 1:1, 2:1 and 4:1 at P+C 180 g l^{-1}, and P:C 1:56, 1:32, 1:16, 1:8, 1:4, 1:2, 1:1 and 2:1 at P+C 270 g l^{-1}. The number of eggs laid on each food patch was counted after 24 h. As mentioned above, because yeast contains diverse nutrients other than protein (e.g. carbohydrates, sterols, fatty acids, minerals and vitamins) (Morais et al., 1995), we standardised its quantity in all food to 10 g l^{-1}. As yeast contains 0.45 g g^{-1} of protein, each diet contained a minimum of 4.5 g l^{-1} of protein. This standardisation prevented us from testing a range of high carbohydrate diets at low P+C concentrations. We conducted 17 to 20 replicates for each P+C concentration and each nutritional state (235 cafeterias; Table S1).

Experiment 5: effect of nutritional state on the interaction between feeding and egg-laying

We examined the feeding and egg-laying preferences of flies maintained on different breeding diets during 96 h. As in experiment 4, flies were transferred to a high carbohydrate diet (P:C 1:16, P+C 180 g l^{-1}), a high protein diet (P:C 8:1, P+C 180 g l^{-1}) or an intermediate diet (P:C 1:2, P+C 180 g l^{-1}) within 2 h of emergence from the pupae, and maintained under these conditions for 96 h. To disentangle the effect of nutritional state on feeding and egg-laying preferences, groups of 10 flies were tested in a cafeteria assay with eight patches of experimental diets, similar to in experiment 2 (P:C 1:16 N=18 groups, P:C 1:2 N=17 groups, P:C 8:1 N=19 groups; Table S1). The number of flies on each diet was recorded every minute using the webcam pictures and the number of eggs laid was counted after 24 h. Nutrient intake was estimated using time spent on food (see details in experiment 3).

Experiment 6: effect of breeding diets on larval growth and survival

To evaluate the consequences of female egg-laying decisions on the fitness of larvae, we measured the development of eggs laid on three different breeding diets. Fifteen groups of five females reared on a standard diet were transferred to culture tubes (55 ml) containing a high carbohydrate diet (P:C 1:16, P+C 180 g l^{-1}), a high protein diet (P:C 8:1, P+C 180 g l^{-1}) or an intermediate diet (P:C 1:2, P+C 180 g l^{-1}) and left to lay eggs for 24 h. The mean number of eggs laid was 34±13 (mean±s.d., N=45 groups), giving us a total of 1518 eggs (Table S1). For all groups, we monitored the time course of larval development from egg to adult emergence by counting the number of pupae and adults on a daily basis over a period of 30 days. Newly emerged adults were removed to prevent females from starting to lay their own eggs.

Experiment 7: effect of breeding diets on larval cognition

To evaluate the consequences of the egg-laying decisions of females on the cognitive abilities of larvae, we measured the learning performances of third instar larvae reared on three different breeding diets using a well-established reciprocal, differential conditioning assay for olfactory learning (Gerber et al., 2013). Fifteen groups of five females reared on a standard diet were transferred to culture tubes (55 ml) containing a high carbohydrate diet (P:C 1:16, P+C 180 g l^{-1}), a high protein diet (P:C 8:1, P+C 180 g l^{-1}) or an intermediate diet (P:C 1:2, P+C 180 g l^{-1}) and allowed to lay eggs for 24 h. Newly hatched larvae were maintained under these conditions until they reached the third stadium (feeding stage).

Groups of 30 larvae underwent one of two reciprocal training assays with 1-octanol (OCT; purity: 99.5%; Sigma-Aldrich) and amyl acetate (AM; purity: 99%, diluted 1:50 in paraffin oil; Sigma-Aldrich). A third of the groups received AM associated with an appetitive sucrose reinforcement and OCT without sucrose (AM+/OCT). A second third of the groups was trained reciprocally (AM/OCT+). The final third was not trained, only tested (control groups). Training arenas (medium Petri dishes, Ø=85 mm, height=20 mm) contained either pure agar gel (1%) or agar gel mixed with sucrose (68.4%). Half of the assays were started with an 'agarose arena', the other half with a 'sucrose arena'. Two containers (1.5 ml Eppendorf tube cap) with the same odorant were placed on opposite sides of the training arena. Training consisted of transferring a group of larvae in the arena and observing them for 5 min. Larvae were then transferred to a second training arena loaded with the alternative odorant and the respective other substrate for 5 min. This cycle was repeated 3 times (6 training trials per

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group). All groups were then tested in a choice condition between AM and OCT without sucrose (AM/OCT) in an agarose arena. We recorded the number of larvae on 'AM' and 'OCT' sides every 30 s for 5 min.

For each assay, we calculated the odour preferences (P) of each group for each time point as the number of larvae on the AM side minus the number on the OCT side, divided by the total number of larvae observed. P ranges from −1 to 1; positive values indicate a preference for AM and negative values indicate a preference for OCT. To determine whether these preferences depended on training, we used the P values from the training assays performed in parallel (AM+/OCT and AM/OCT+) and computed a learning index (LI):

$$\mathrm{LI} = \frac{P(\mathrm{AM+,OCT}) - P(\mathrm{AM,OCT+})}{2}. \quad (3)$$

LI ranges from −1 to 1; positive values indicate associative learning between the odorant and the sucrose reinforcement. We tested 30 groups for each nutritional treatment (P:C 1:16, 1:2, 8:1). Ten groups were trained with AM+/OCT, 10 groups with AM/OCT+ and 10 groups were the naïve controls (Table S1).

Statistical analyses

For experiment 1, we used Lande–Arnold regressions to estimate parametric nonlinear response surfaces. These comprise linear and quadratic components for protein and carbohydrate concentrations and the cross-product of both nutrients. Response surfaces for number of eggs laid were fitted over P:C intake. These surfaces are best visualised using non-parametric techniques that do not constrain the shape of the surface. We fitted non-parametric thin-plate splines using the 'fields' package in R (http://CRAN.R-project.org/package=fields).

All other analyses were conducted with SPSS (v21.0). For experiments 2–5, we used generalised linear mixed models (GLMM) with a binomial logit function to compare the oviposition preferences. The number of individuals (experiment 2), behaviour (feeding or egg-laying; experiment 3), nutritional state (experiments 4 and 5), nutrient concentration (experiment 5) and nutrient ratio (experiments 2–5) of diets were added as fixed factors; the total number of eggs was added as a covariate; and the cafeteria replicate was added as a random factor. We used general linear models (GLM) to compare the total number of eggs laid in each cafeteria assay with nutritional state (experiments 4 and 5) and nutrient concentration of diets (experiment 5) as fixed factors.

For experiment 6, we used a GLM to compare larval development time in relation to the nutritional state and a GLM with a logit function to compare the proportion of adult emergence in relation to the nutritional state. In both models, nutrient ratios of diets were used as a fixed factor and group of flies as a nested factor.

For experiment 7, we used a GLMM to investigate the effect of the nutritional state on the cognitive performances of larvae. Time was used as a within-subject factor and diet as a between-subject factor.

RESULTS

Experiment 1: egg-laying performance

Flies confined to one of 34 foods varying in nutrient balance and concentration laid more eggs on high carbohydrate foods (R^2=0.19, $F_{5,806}$=38.17, P<0.001; Table S2). The number of eggs peaked on P:C 1:8 (Fig. 1). This number decreased sharply with increasing ratio and concentration of protein, reaching a minimum on P:C 8:1.

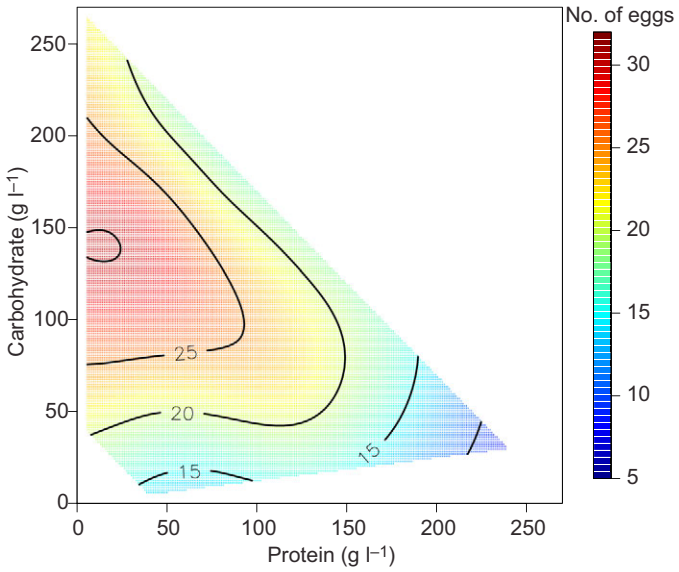

Fig. 1. Egg-laying performances in no-choice assays (experiment 1). Effects of nutrient balance and concentration on the number of eggs laid by individual flies confined for 24 h to one of 34 diets (N≥20 flies for each diet; Table S1). Response surfaces were generated using non-parametric thin-plate splines fitted using the 'fields' package in R (http://CRAN.R-project.org/package=fields) (see details of Lande–Arnold regression in Table S2). Dark red indicates the highest values of the number of eggs laid, descending to the lowest values in dark blue regions.

Experiment 2: egg-laying preferences

When offered a choice between eight foods varying in nutrient balance at stable concentration, flies consistently showed an oviposition preference for high carbohydrate foods, laying the majority of their eggs on P:C 1:16 and P:C 1:8 (GLMM, diet: $F_{7,496}$=53.10, P<0.001; Fig. 2). The number of eggs increased with decreasing P:C ratio in a similar manner for flies tested in isolation or in groups. However, the preference for high carbohydrate diets was more pronounced in grouped flies (GLMM, social condition: $F_{7,496}$=29.65, P<0.001; diet×social condition: $F_{7,496}$=7.87, P<0.001; Fig. 2). Therefore, we conducted all the following choice experiments (experiments 3–5) with groups of flies.

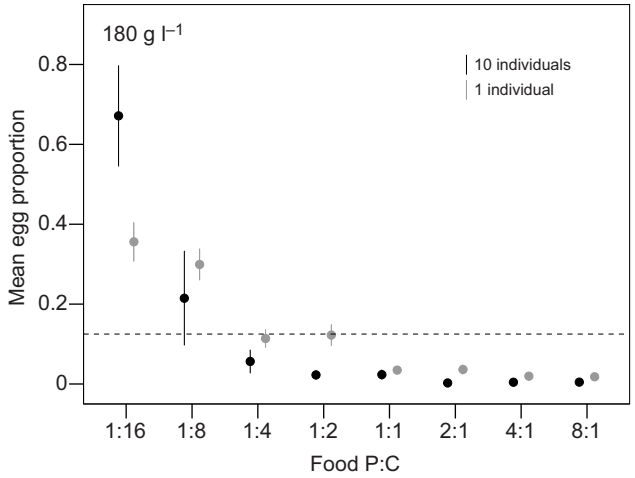

Fig. 2. Egg-laying preferences in choice assays (experiment 2). Mean proportion of eggs laid on each food patch for individual flies (N=40 flies) or groups of flies (N=24 groups of 10; Table S1). The dashed line indicates random choice. Error bars indicate ±95% CI.

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Experiment 3: interaction between feeding and egg-laying preferences

Detailed analyses of the choice dynamics by groups of flies in cafeteria assays confirmed the results of experiment 2 that females spent most of their time and laid most of their eggs on high carbohydrate foods (GLMM, diet: $F_{7,320}$=31.14, P<0.001; Fig. 3). If we consider that it takes 1 min for each fly to lay one egg, egg-laying represented a maximum of 20% of the time spent on the high carbohydrate foods. This suggests that flies also visited these foods for feeding. However, the mean proportion of flies observed on the different foods was not perfectly correlated with the mean number of eggs laid, suggesting that feeding decisions and oviposition decisions were uncoupled to some extent. On average, flies spent 23% of the time (N=21 groups) on high protein foods (P:C 2:1, 4:1, 8:1) while not laying eggs on them (GLMM, behaviour: $F_{7,320}$=21.42, P<0.001; diet×behaviour: $F_{7,320}$=9.01, P<0.001; Fig. 3). Our estimations of protein and carbohydrate intake (based on total number of flies observed on foods) suggest that flies acquired both nutrients at a P:C ratio of 1:1.6 (R^2=0.88, $F_{1,21}$=155.15, P<0.001; Fig. 4).

Experiment 4: effect of nutritional state on egg-laying performances and preferences

Manipulation of the nutritional state of flies fed different breeding diets for 96 h induced important changes in their feeding and egg-laying behaviour. In no-choice conditions, flies confined to standard diet laid more eggs when fed high protein diet P:C 8:1 than when fed balanced diet P:C 1:2 or high carbohydrate diet P:C 1:16 (GLM, nutritional state: $F_{2,40}$=67.97, P<0.001; Fig. 5A). Flies offered a choice between eight food patches varying in nutrient balance and concentration laid more eggs when fed high protein P:C 8:1, than when fed balanced P:C 1:2 or high carbohydrate P:C 1:16 diets (GLM, nutritional state: $F_{2,235}$=555.21, P<0.001; Fig. 5B). This difference in egg production was more pronounced in cafeteria assays with high nutrient concentrations (concentration: $F_{3,235}$=30.31, P<0.001; concentration×nutritional state: $F_{6,235}$=31.69, P<0.001; Fig. 5B). For all P+C concentrations, flies laid the majority of their eggs on foods with a carbohydrate-biased P:C ratio (GLMM, 45 g l^{-1}: $F_{7,448}$=192.77, P<0.001; 90 g l^{-1}: $F_{7,448}$=208.18, P<0.001; 180 g l^{-1}: $F_{7,448}$=429.40, P<0.001; 270 g l^{-1}: $F_{7,448}$=289.21; Fig. 6), thereby confirming the results of experiments 2–4. However, the choice became more significant and specific to foods with the highest carbohydrate ratio (P:C 1:56) when the nutrient concentration was increased. Presumably the presence of nutrients in higher concentrations facilitated the discrimination between close P:C ratios by flies.

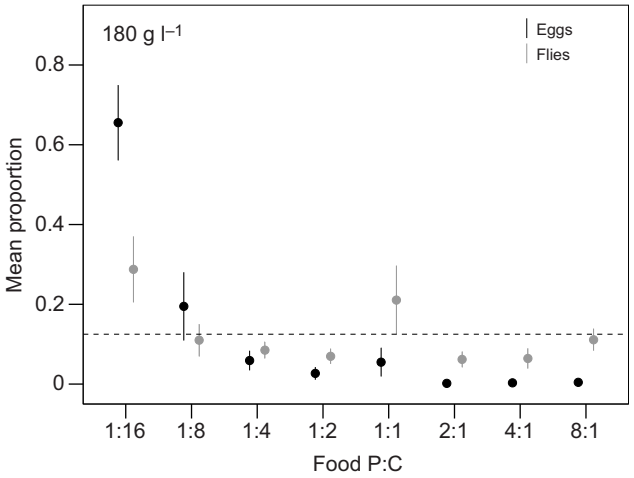

Fig. 3. Interaction between feeding and egg-laying in choice assays (experiment 3). Mean proportion of flies and eggs on each diet (N=21 groups of 10 flies; Table S1). The dashed line indicates random choice. Error bars indicate ±95% CI.

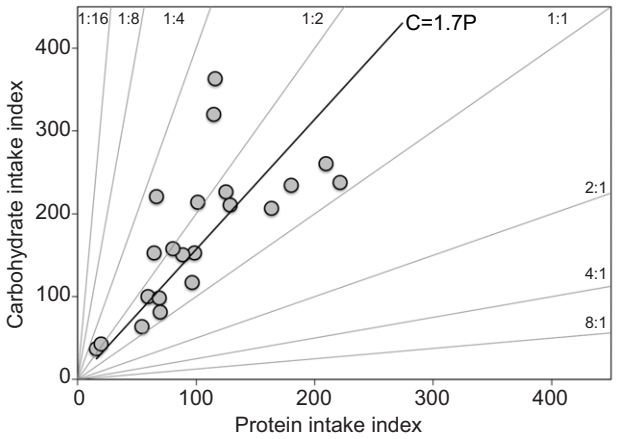

Fig. 4. Estimation of nutrient intake (experiment 3). Indices of protein and carbohydrate intake computed from the time spent on each of the eight foods (N=21 groups of 10 flies; Table S1). Grey lines represent foods. Slope indicates the C:P ratio of each food.

Experiment 5: effect of nutritional state on the interaction between feeding and egg-laying

When given a choice between eight foods varying in nutrient balance, flies laid more eggs on high carbohydrate food (P:C 1:16), regardless of their nutritional state, thus confirming the result of experiment 2 (GLMM, diet: $F_{7,408}$=57.93, P<0.001; nutritional state: $F_{2,408}$=1.12, P=0.333; Fig. 7). Although the total number of eggs laid on all foods was much higher in flies fed high protein diet P:C 8:1 (GLM, nutritional state: $F_{2,53}$=67.97, P<0.001; Fig. S1), the total number of flies observed on all foods did not differ according to their nutritional state (GLM, nutritional state: $F_{2,53}$=2.74, P=0.074). The distribution of flies across the different foods, however, varied considerably with nutritional state (Fig. 7). Flies fed P:C 8:1 were observed on both P:C 1:16, while flies fed P:C 1:2 and P:C 1:16 were observed on both P:C 8:1 and P:C 1:16 (GLMM, diet: $F_{7,408}$=96.12, P<0.001; nutritional state: $F_{2,408}$=7.26, P=0.001; diet×nutritional state: $F_{14,408}$=5.05, P<0.001; Fig. 7). Our estimations of protein and carbohydrate intake (based on cumulated time spent on foods) suggest that flies acquired both nutrients at varying P:C ratios depending on their nutritional state (P:C 1:3.8, P:C 1:1.6 and P:C 1:1.4 for 8:1, 1:2 and 1:16 nutritional states, respectively; Fig. 8). Overall, flies fed high carbohydrate diets spent more time on high protein foods, while flies fed high protein diets spent more time on high carbohydrate foods. These opposite behavioural responses by flies with divergent nutritional states indicate a strategy of compensatory feeding (illustrated in Fig. 9).

Experiment 6: effect of breeding diets on larval growth and survival

The nutrient content of breeding diets had a considerable effect on larval development (Fig. S2). Larvae had the fastest egg-to-adult development on P:C 8:1 and the slowest egg-to-adult development on P:C 1:16 (GLM, diet: $F_{1,44}$=38.34, P<0.001; mean±s.d., P:C

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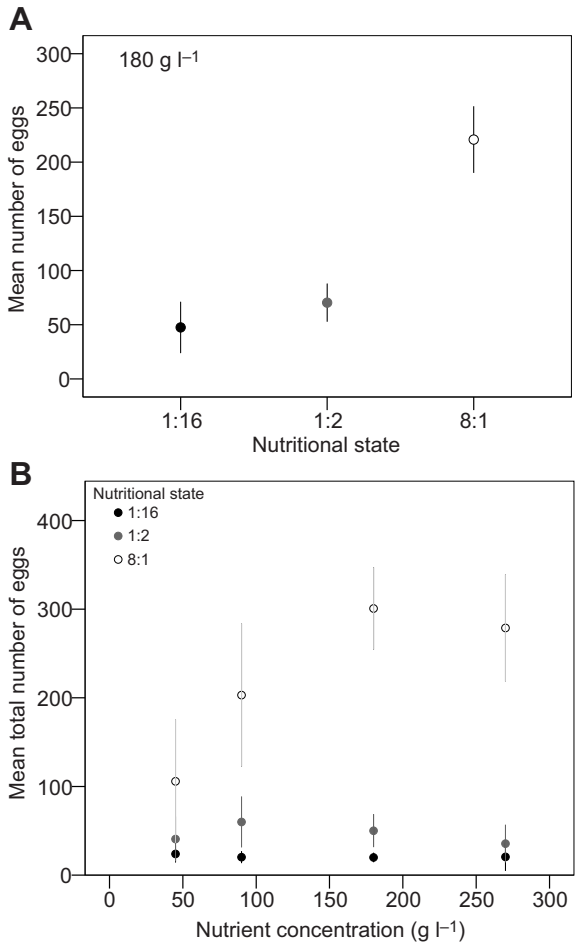

Fig. 5. Effect of nutritional state on egg-laying preference: mean total number of eggs according to the nutritional state of flies (experiment 4). (A) Egg-laying performance in no-choice assays. Mean total number of eggs laid on a standard diet according to the nutritional state of flies (*N*=40 flies for each nutritional state). (B) Egg-laying preference in choice assays. Mean total number of eggs laid in each cafeteria according to the nutritional state of flies (*N*=79 groups for P:C 1:16, *N*=79 groups for P:C 1:2, *N*=77 for P:C 8:1; Table S1) and P+C concentration of foods in each cafeteria (*N*=60 groups for 45 g l^{-1}, *N*=59 groups for 90 g l^{-1}, *N*=59 groups for 180 g l^{-1}, *N*=57 for 270 g l^{-1}). Flies were tested in groups of 10. Error bars indicate ±95% CI.

1:16=22.5±2.8 days, P:C 1:2=17.3±2.5 days, P:C 8:1=14.3±2.1 days). However, the proportion of adults that successfully emerged from pupae was the lowest on P:C 8:1 and the highest on P:C 1:2 (GLM, $\chi^2_{1,44}$=204.55, *P*<0.001, proportion of emergence: P:C 1:16=0.63, P:C 1:2=0.74, P:C 8:1=0.47). Thus, overall, the developmental performance of larvae (combining growth and survival) was the highest on P:C 1:2.

Experiment 7: effect of breeding diets on larval cognition

The larvae belonging to the naïve control group did not express any innate preference for either of the odours during the test (mean proportion of the larvae observed on the AM side ±95% CI, P:C 1:16=0.51±0.07, P:C 1:2=0.55±0.05, P:C 8:1=0.51±0.06; Fig. S3). However, the composition of breeding diets impacted on the cognitive capacities of larvae, influencing both their learning performances and decision speed. Overall, larvae fed P:C 1:2 showed higher learning indices than larvae fed P:C 1:16 and larvae fed P:C 8:1 (GLM, nutritional state: $F_{1,27}$=4.01, *P*=0.03; Fig. 10). During the test trials, larvae fed P:C 1:16 showed the shortest latency (60 s to reach a plateau) to join the side scented with the reinforced stimulus, either AM+ or OCT+, while larvae fed P:C 8:1 showed the longest latency (240 s to reach a plateau) (GLM, time: $F_{9,243}$=43.31, *P*<0.001; time×breeding diet: $F_{18,243}$=3.69, *P*<0.001). Thus, the overall associative olfactory learning performance of larvae (combining the decision speed and accuracy during the test) was the highest on P:C 1:2.

DISCUSSION

We sought to understand how female fruit flies integrate feeding decisions (individual nutrition) and oviposition decisions (offspring nutrition) in their foraging activities, and how these trade-offs impact the fitness of the future larvae. Our observation of time spent on foods and egg counts indicate that flies exhibit complex foraging patterns during which they alternate between feeding on balanced diets known to maximise female fitness and laying eggs on high carbohydrate diets that are suboptimal for larval development. The apparent mismatch between the oviposition choices of females and the nutritional requirements of larvae may reflect a natural situation where ripening (high carbohydrate) fruits gradually enrich in protein as they start rotting, thereby providing good nutrition for the developing larvae.

Deciding where to feed and where to lay eggs are critical nutritional decisions for *D. melanogaster* females and their progeny. In all our different choice experiments, eggs were almost exclusively observed on high carbohydrate diets (P:C 1:16 and 1:8) irrespective of the nutritional state of flies. Selectivity for oviposition sites rich in carbohydrates is consistent with previous observations that *D. melanogaster* females given a simultaneous choice between multiple foods prefer laying eggs on sucrose substrates (Schwartz et al., 2012) or on mixed-sugar protein substrates with high carbohydrate ratios (Rodrigues et al., 2015) over yeast media. Interestingly, we found that these choices were more pronounced in groups than in isolated females. Presumably, aggregation on foods mediated by social information transfer between foraging flies (e.g. phenomenal cues such as cis-11-vaccenyl acetate or sex-specific cuticular hydrocarbons) increased the accuracy of their oviposition decisions (Duménil et al., 2016; Lihoreau et al., 2016; Philippe et al., 2016), a well-known property of collective decision-making in animal groups (Couzin, 2009).

Monitoring of the complete foraging patterns of flies over 24 consecutive hours revealed that females alternated visiting diets with distinct nutrient contents. This pattern is incompatible with the hypothesis that flies simply lay eggs where they eat. Instead, females clearly engaged in a complex succession of nutritional decisions to simultaneously self-regulate their own nutrient intake while also searching for suitable nutritional habitats for the future larvae, a foraging pattern that we do not expect to observe in virgin or sterile females. Females reared on a standard food were mostly seen on the balanced diet (P:C 1:1), reaching an estimated intake target of P:C 1:1.6. This estimation is similar to recent measures of intake targets by *D. melanogaster* based on actual consumption of liquid foods (Lee et al., 2013). Accordingly, females reared on an imbalanced diet P:C 1:16 (or P:C 8:1) were more often observed on a nutritionally complementary diet P:C 8:1 (or P:C 1:16), possibly in an attempt to compensate for their deficiency of one of the two nutrients. The pattern of food visitations combined with egg-laying performances show that flies need protein to lay eggs, confirming previous observations that egg production is related to both available nutrients and the nutritional state of females in *D. melanogaster* and many other insects (Rivero et al., 2001; Terashima and Bownes, 2004). Flies reared on high carbohydrate

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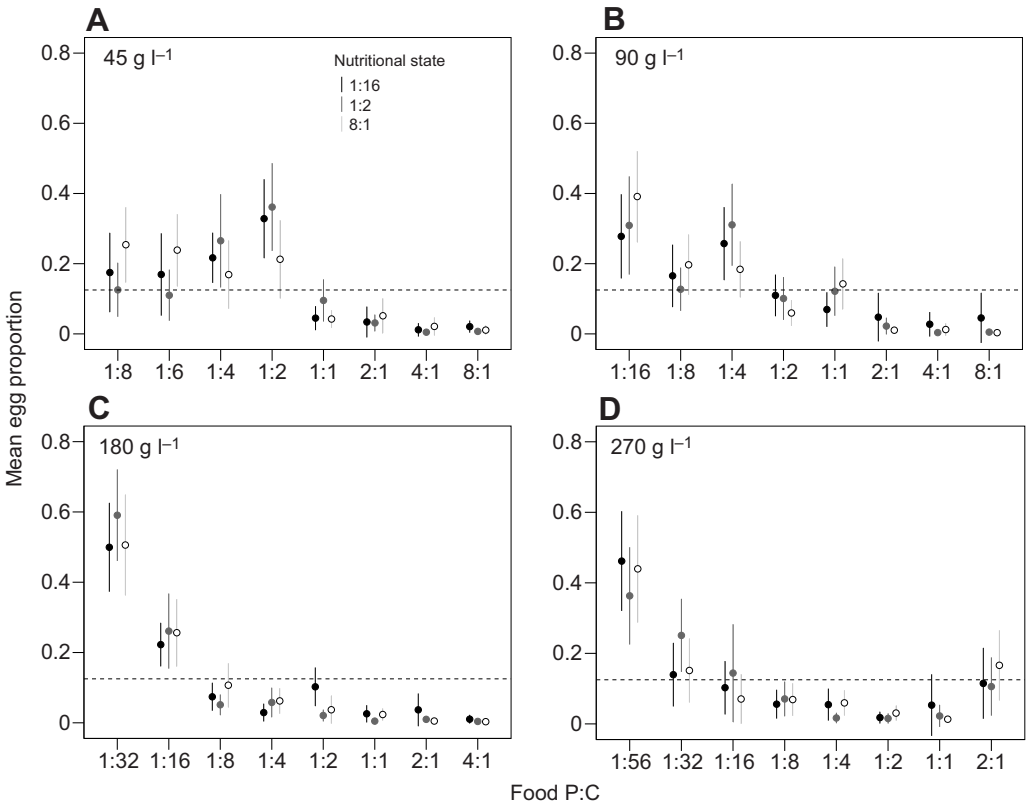

Fig. 6. Effect of nutritional state on egg-laying preference in choice assays (experiment 4). Mean proportion of eggs laid on each food according to the nutritional state of flies (*N*=79 groups for P:C 1:16, *N*=79 groups for P:C 1:2, *N*=77 groups for 8:1) and P+C concentration of foods: (A) *N*=60 groups for 45 g l^{-1}, (B) *N*=59 groups for 90 g l^{-1}, (C) *N*=59 groups for 180 g l^{-1} and (D) *N*=57 groups for 270 g l^{-1}. Flies were tested in groups of 10. The dashed lines indicate random choice. Error bars indicate ±95% CI.

diet (P:C 1:16) laid few eggs and visited high protein diets as soon as they were introduced in the cafeteria assay. In contrast, flies reared on high protein diet (P:C 8:1) laid numerous eggs and visited high protein diets only later, towards the end of the experiment. These results thus confirm that when given a choice between complementary foods, *D. melanogaster* mated females exhibit compensatory feeding, which enables them to balance their intake of protein and carbohydrates to reach nutritional states maximising egg production (Lee et al., 2008, 2013; Piper et al., 2014; Ribeiro and Dickson, 2010).

Our analyses of the performances of larvae confined to specific diets show that development was impaired on high carbohydrate diets (P:C 1:16), as illustrated by the 15% decrease in survival, 30% increase in egg-to-adult development duration and 30% reduced

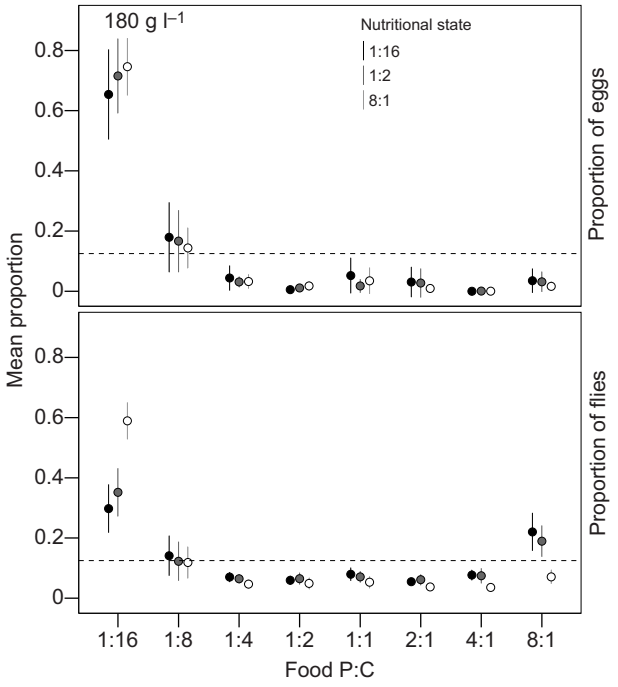

Fig. 7. Effect of nutritional state on the interaction between feeding and egg-laying in choice assays (experiment 5). Mean proportion of eggs and flies on each food according to the nutritional state of flies (*N*=18 groups for P:C 1:16, *N*=17 groups for P:C 1:2, *N*=19 groups for P:C 8:1). The P+C concentration was 180 g l^{-1}. Flies were tested in groups of 10. The dashed lines indicate random choice. Error bars indicate ±95% CI.

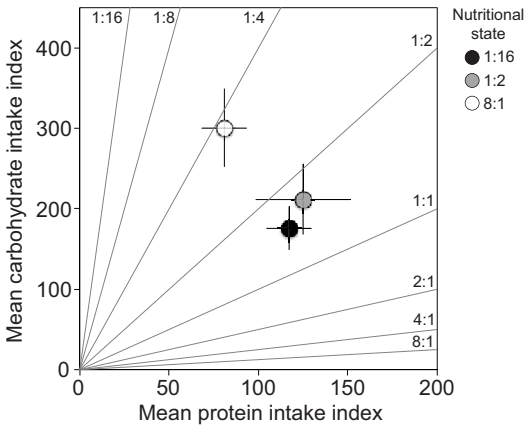

Fig. 8. Effect of nutritional state on nutrient intake (experiment 5). Indices of protein and carbohydrate intake computed from the time spent on foods according to the nutritional state of flies (*N*=18 groups for P:C 1:16, *N*=17 groups for P:C 1:2, *N*=19 groups for P:C 8:1). Flies were tested in groups of 10. Grey lines represent foods. Slope indicates the C:P ratio of each food. Bivariate error bars indicate ±95% CI.

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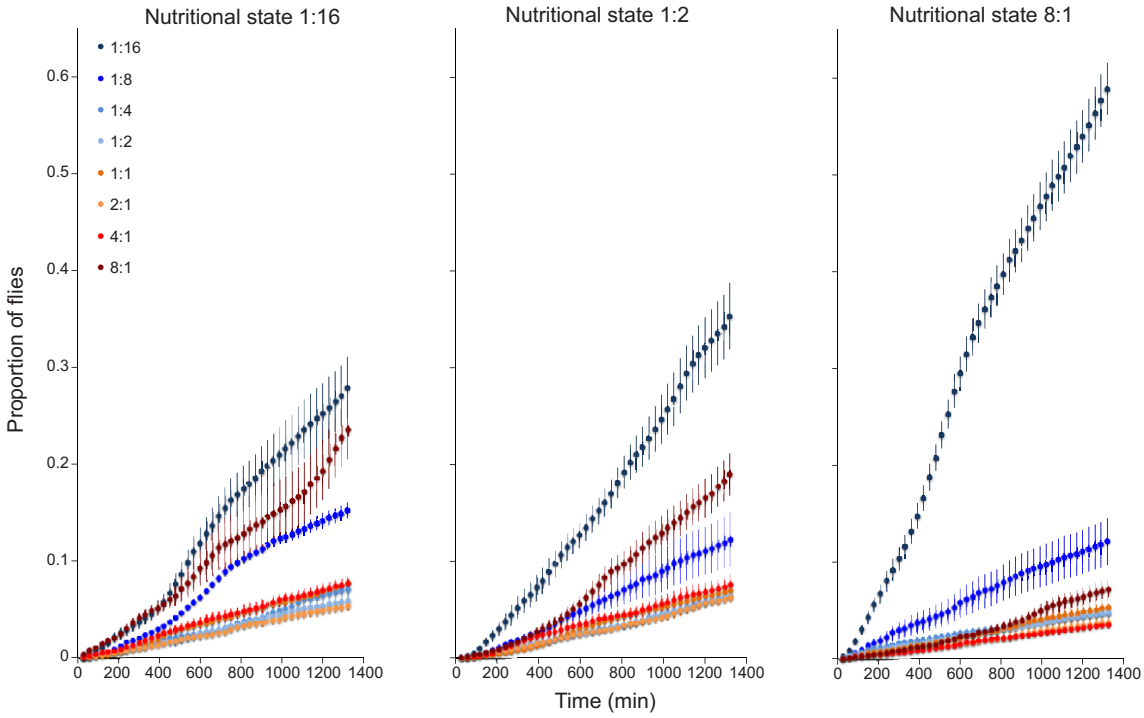

Fig. 9. Effect of nutritional state on food visitation dynamics (experiment 5). Cumulative proportion of flies observed on each food (*N*=18 groups for P:C 1:16, *N*=17 groups for P:C 1:2, *N*=19 groups for P:C 8:1). Flies were tested in groups of 10. The colour code indicates variance in P:C ratios, from high carbohydrate diets (dark blue) to high protein diets (dark red). Error bars indicate ±95% CI.

learning scores in comparison to flies reared on more balanced diets. The highest larval performances were obtained for flies raised on P:C 1:2, which is consistent with recent estimates of the *D. melanogaster* larval nutrient intake target (Rodrigues et al., 2015). Accordingly, the worst performances were observed for flies raised on P:C 8:1, with only half of the larvae reaching the imaginal moult, suggesting that protein overconsumption has a toxic effect on larvae, as previously demonstrated in adult insects [e.g. *Drosophila* (Lee et al., 2008), ants (Dussutour and Simpson, 2012), bees (Stabler et al., 2015) and field crickets (Maklakov et al., 2008)]. Alternately, it is possible that a hard ceiling on protein intake slowed food consumption so that larvae actually suffered from a lethal carbohydrate deficit (Simpson and Raubenheimer, 2005; Felton et al., 2009).

Importantly, we found that learning performances are also directly affected by diet, thereby adding a new dimension to the fitness landscape of *D. melanogaster* larvae. The effects of malnutrition on cognitive performances have long been identified in mammals (La Rue et al., 1997) and insects [e.g. honeybees (Arien et al., 2015; Wright et al., 2013) and *Drosophila* (Guo et al., 1996; Kawecki, 2010; Kolss and Kawecki, 2008; Shou-Zhen et al., 1997)], and may be due to modifications of the biochemical composition of the brain, developmental procedures (Heisenberg et al., 1995; Xia et al., 1997) or sensorial modalities (e.g. impaired olfaction). Previous studies on fruit flies indicate that adults fed high carbohydrate diets (ca. P:C 1:12) have reduced performances in operant visual learning tasks (Guo et al., 1996; Shou-Zhen et al., 1997). However, none of these studies have systematically compared the cognitive performances of flies fed diets varying in their contents of specific nutrients. Our results indicate that a diet balanced in protein and carbohydrate is critical for learning. Our future experiments using more diets to cover the entire nutrient space will determine whether impairment of learning is caused by an excess and/or deficit of one nutrient or both. In the case of *D. melanogaster* larvae, learning associations between odours and food rewards may be of primary importance for guiding their foraging decisions in the dark (Schleyer et al., 2015). Within a single rotting fruit, the stochastic nature of colonisation by bacteria and fungi may lead to considerable spatio-temporal variation of nutrient distribution, providing patchy and ephemeral foraging environments (Reaume and Sokolowski, 2006). Olfactory learning may therefore be useful for larvae to accurately navigate between

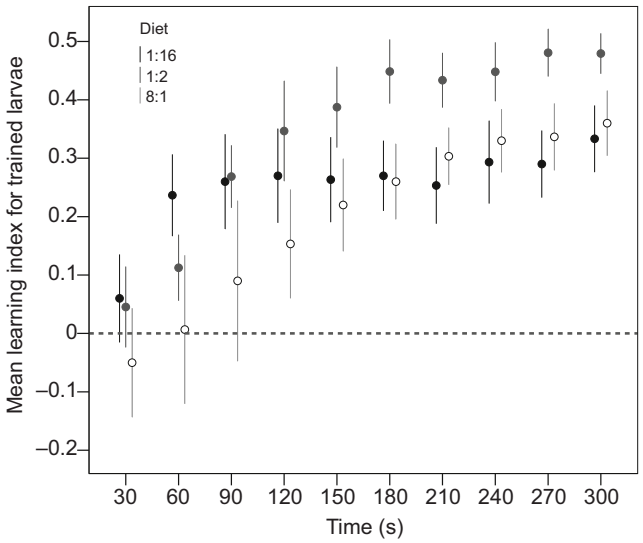

Fig. 10. Effect of nutritional state on larval cognition (experiment 7). Learning index (LI) according to the nutritional state of flies (*N*=10 groups of 30 larvae for each nutritional state). LI ranges from –1 to 1. Positive values indicate successful associative learning. Error bars indicate ±95% CI.

patches of nutritious substrates interspaced with non-nutritious areas free of microbes.

The apparent mismatch between female egg-laying preferences and larval performances suggests that flies integrate the gradual dynamics of fruit decomposition in their egg-laying decisions. In nature, as a fruit starts rotting and yeast populations grow, the composition of the fruit dynamically enriches in protein, thus providing food resources with increased P:C ratios (Matavelli et al., 2015; Morais et al., 1995). For instance, the composition of a ripening fig fruit changes from ca. P:C 1:10 to P:C 10,000:1 over the course of 27 days, with P and C concentrations varying between 10 and 10,000 g l^{-1} (Matavelli et al., 2015). These nutritional modifications of food resources are likely favoured by the fact that females inoculate the fruit substrate with yeast during oviposition (Buser et al., 2014; Stamps et al., 2012) and tend to lay eggs in aggregations (Navarro and del Solar, 1975; Prokopy and Roitberg, 2001; Wertheim et al., 2005; see also experiment 2). In many cases, multiple fly species may also breed in the same fruits (Matavelli et al., 2015). These changes in nutrient balance and concentration mediated by the behaviour of females correlate with changes in the nutrient requirements of larvae as they develop. Thus under this hypothesis, foods with a high P:C ratio may indicate a stage in the food decay process that is too advanced to sustain the development of the larvae and, therefore, a poor-quality oviposition site. This is consistent with observations that *D. melanogaster* females prefer laying eggs in fruits with intermediate levels of decay (Hoffmann, 1985) and that these preferences vary among drosophilid species (Matavelli et al., 2015). Such selectivity for an optimal nutrient mix may explain some of the variance observed in female oviposition choices under laboratory conditions, where the preference for a non-nutritious substrate (medium without yeast) changes depending on its distance to a nutritious substrate (medium containing yeast) (Miller et al., 2011), presumably because flies use gradients of nutrient concentration rather than discrete food patches for selecting appropriate sites. Future experiments with nutritional geometry designs to measure how the intake targets of larvae may change throughout their development are needed to definitively answer this question (Simpson and Raubenheimer, 1993).

Although ample evidence shows that early diet can have critical consequences on the physiology and behaviour of animals (Simpson and Raubenheimer, 2012), the nutritional ecology of parent–offspring interactions has so far received little attention. Our study, in a model organism for nutrition research with minimal parental care, reveals that females combine their own nutritional regulation with complex oviposition decisions, anticipating changes in food nutrient contents in their foraging activities. Although it is clear from our results that these nutritional and oviposition decisions are independent from each other (at least partially), it is possible that longer-term dietary experiences may affect the egg-laying behaviour of females. For instance, it has been proposed that *D. melanogaster* females can adjust their investment in offspring based on the quality of the nutritional environment, so that flies bred on poor diets (low P+C) produce higher quality offspring (e.g. heavier eggs, faster larval development, higher reproductive output) than flies bred on rich diets (high P+C) to maximise their chance of surviving (Matzkin et al., 2013; Vijendravarma et al., 2010). Similarly, it is possible that a long-term exposure to an unbalanced diet (nutritional stress) causes females to lay eggs on nutritionally complementary diets in order to anticipate protein compensation by the larvae.

Future studies are needed to explore how these complex alloregulatory behaviours are adjusted in relation to the nutritional context across taxa and socio-ecological environments. In nature, nutritional decisions can be complicated by several additional factors such as social information provided by other females (Battesti et al., 2012; Durisko et al., 2014; Lihoreau et al., 2016; Sarin and Dukas, 2009; Chabaud et al., 2009), competition (Eggert et al., 2008; Salomon et al., 2008), sexual interactions with males (Chapman and Partridge, 1996; Gorter et al., 2016) or the presence of beneficial microbial communities on foods (Venu et al., 2014; Wong et al., 2015). Thanks to their unique association with food as shelter, breeding sites and sources of nutrients, fruit flies hold considerable promise as model organisms with which to study these multi-level nutritional interactions within the extended integrative framework of nutritional ecology (Simpson et al., 2015a).

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Competing interests
The authors declare no competing or financial interests.

Author contributions
A.D. and M.L. designed the study. L.-A.P. performed the research. A.D. and M.L. analysed the data. A.D. and M.L. wrote the first draft of the manuscript, and all authors contributed to revisions. All authors gave final approval for publication.

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Supplementary information
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