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Title: *Drosophila* females trade off good nutrition with high quality oviposition sites when choosing foods

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Summary statement: Flies uncouple feeding and egg-laying decisions to balance their diet and provide a nutritionally optimal environment to their progeny, indicating a certain complexity in the nutritional ecology of parent-offspring interactions.

Abstract

Animals, from insects to human, select foods to regulate their acquisition of key nutrients in amounts and balances maximising fitness. In species where 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 the progeny. Here we examined how fruit flies *Drosophila melanogaster*, a species where 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 *Drosophila* 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 performances 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 reveals 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; Simpson and Raubenheimer, 2012; Simpson et al., 2015a; Simpson et al., 2015b). 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; Lihoreau et al., 2015; Senior et al., 2015; Senior et al., 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 in these conditions an individual's decision to eat a food not only depends 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 optimizing 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 the 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; Dussutour and Simpson, 2012), honeybees (Altaye et al., 2010; Hendriksma and Shafir, 2016), bumblebees (Stabler et al., 2015)).

Although most research on dietary allo-regulation (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 where 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 where animals lay eggs in food resources, such as the 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 the offspring (Reaume and Sokolowski, 2006). Since fruit fly larvae have limited mobility, their nutrition is largely determined by their mother's choice of oviposition site, making egg-laying decisions crucial for the survival of embryos and larvae.

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Recent studies using nutritional geometry, a conceptual approach to dissect the nutritional interactions between animals and their environment (Simpson and Raubenheimer, 1993; Simpson and Raubenheimer, 2012; Simpson et al., 2015b), have showed 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; Lee et al., 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 the physiology and behaviour greatly vary with age, sex, and the mating status of individuals. For instance when provided with nutritionally 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; Lee et al., 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 *Drosophila* 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 *Drosophila* 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 with 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. 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 their deficit in carbohydrates (Lee et al., 2008; Lee et al., 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 the 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.

Methods

Fly culture: Wild-type Canton-S *D. melanogaster* flies (Bloomington *Drosophila* stock center) were reared in standard conditions (20°C, 60% relative humidity, 12:12 L:D photoregime, light on at 8:00 am). Flies were cultured in 150 mL plastic bottles containing standard diet made of dry inactive yeast (90 $g.L^{-1}$, Dutscher), maize flour (90 $g.L^{-1}$, Genesee Scientific), Vanderzant vitamin mixture for insects (2.5 $g.L^{-1}$, Sigma), Tegosept (4 $g.L^{-1}$, Dutscher) and propionic acid (1.5 $g.L^{-1}$, Dutscher) in a 1.5% agar gel (Dutscher). The protein to carbohydrate ratio of the standard diet was P:C 1:2.

Experiments 1- 7 were conducted with four-day-old mated females. To obtain mated females, virgin adults were collected in the stock culture within 2h 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 96h, females were transferred in a test arena (experiments 1-5) or in plastic tubes (experiments 6-7) under light CO_2 anesthetisia (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) that is not detected by flies (Heisenberg and Buchner, 1977). All experiments were started at 10:00 am. 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). The carbohydrate content was manipulated using sucrose (Dutscher). The quantity of yeast (dry and inactive, Dutscher) 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), Tegosept (4 $g.L^{-1}$, Dutscher) and propionic acid (1.5 $g.L^{-1}$, Dutscher) were added to each

diet. All diets were presented to the insects in a 2 % agar gel (Dutscher), providing both 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, 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, 8:1). Each fly was tested for 24h in a small petri dish (Ø=35mm, H=15mm) filled with 5mL 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 24h, during which they had unrestricted access to eight patches of different experimental diets (Ø=35mm, H=15mm, V=5mL) set in a 15 mL agar gel basis ($30g.L^{-1}$) in large petri dish (Ø=145mm, H=20mm). Diets varied in their nutrient ratios (P:C 1:16, 1:8, 1:4, 1:2, 1:1, 2:1, 4:1, 8:1, P+C $180g.L^{-1}$, 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 hours. Top view pictures of the test arena were taken every minute with a webcam (Logitech HD Webcam C270) placed 150mm above the setup and programmed with Zone Trigger (Omega Unfold). The number of flies on each food patch was counted on every 80640 images recorded using the "analyse particles" tool in ImageJ (National Institute of Health) [see details of the image analysis procedure in (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:

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

$$I_C = \sum_{t=1}^{x} \sum_{i=1}^{8} \frac{T_i \times C_i}{N}$$

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Where t is the time since the beginning of the experiment (0 to 1440 minutes), N is the number of flies in the cafeteria, T_i is the cumulated time spent on food patch i, 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 of 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 a minute (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 180g.L^{-1}), a high protein diet (P:C 8:1, P+C 180g.L^{-1}), or an intermediate diet (P:C 1:2, P+C 180g.L^{-1}) within 2h of emergence from the pupae, and maintained in these conditions for 96h. We used a first batch of flies to investigate the role of nutritional state on 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 on 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 360g.L^{-1}. The following cafeterias were used: P:C 1:8, 1:6, 1:4, 1:2, 1:1, 2:1, 4:1, 8:1 at P+C 45g.L^{-1}; P:C 1:16, 1:8, 1:4, 1:2, 1:1, 2:1, 4:1, 8:1 at P+C 90g.L^{-1}; P:C 1:32, 1:16, 1:8, 1:4, 1:2, 1:1, 2:1, 4:1 for P+C 180g.L^{-1}, and P:C 1:56, 1:32, 1:16, 1:8, 1:4, 1:2, 1:1, 2:1 at P+C 270g.L^{-1}. The number of eggs laid on each food patch was counted after 24 hours. As mentioned above, because yeast contains diverse nutrients other than protein (e.g. carbohydrates, sterols, fatty acids, minerals, vitamins) (Morais et al., 1995), we standardised its quantity in all food to 10g.L-1. As yeast contains 0.45g.g-1 of protein, each diet contained a minimum of 4.5g.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 96h. Like in experiment 4, flies were either transferred to a high carbohydrate diet (P:C 1:16, P+C 180g.L^{-1}), a high protein diet (P:C 8:1, P+C 180g.L^{-1}), or an intermediate diet (P:C 1:2, P+C 180g.L^{-1}) within 2h of emergence from the pupae, and maintained in these conditions for 96h. In order 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 in culture tubes (55mL) containing a high carbohydrate diet (P:C 1:16, P+C 180g.L^{-1}), a high protein diet (P:C 8:1, P+C 180g.L^{-1}) or an intermediate diet (P:C 1:2, P+C 180g.L^{-1}) to lay eggs for 24h. The mean number of eggs laid was 34 ± 13 (mean ± SD, 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 during a period of 30 days. Newly emerged adults were removed to prevent females to start laying 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 in culture tubes (55mL) containing a high carbohydrate diet (P:C 1:16, P+C 180g.L^{-1}), a high protein diet (P:C 8:1, P+C 180g.L^{-1}) or an intermediate diet (P:C 1:2, P+C 180g.L^{-1}) to lay eggs for 24h. Newly hatched larvae were maintained in 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=85mm H=20mm) 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 in 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 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. 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):

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

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 visualized by using non-parametric techniques that do not constrain the shape of the surface. We fitted non-parametric thin- plate splines using the *fields* R package (Nychka et al., 2016).

All other analyses were conducted with SPSS (v21.0). For experiments 2, 3, 4 and 5 we used generalised linear mixed models (GLMM) with a binomial logit function to compare the oviposition preferences. The number of individuals (experiment 2), the behaviour – feeding or egg-laying – (experiment 3), the nutritional state (experiments 4 and 5), the nutrient concentration (experiment 5), and the nutrient ratio (experiments 2-5) of diets were added as fixed factor; 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 within subject factor and diet as a between subject factor.

Results

Experiment 1: Egg-laying performances

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.

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 C 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.

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 one minute 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 visited these foods also 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 ratio P:C 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 96h 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 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 x 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 (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 concentration facilitated the discrimination between close P:C ratios by flies. .

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 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 mostly observed on P:C 1:16, while flies fed P:C 1:2 and P:C 1:16 were mostly observed on 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 x 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, 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. 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±SD P:C 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, $X^2_{1,44}$= 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 one of the odours during the test (mean proportion of the larvae observed on the AM side ±CI95, P:C 1:16 = 0.51±0.07, P:C 1:2 = 0.55±0.05, P:C 8:1 = 0.51±0.06). 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 to join the side scented with CS+ (60 s to reach a plateau) 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 x 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 on 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 *Drosophila* females and their progeny. In all our different choice experiments, eggs were almost exclusively observed on high carbohydrate diets (P:C 1:16 and P:C 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 a sucrose substrates (Schwartz et al., 2012) or on a 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 between 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 food P:C 1:16 (or P:C 8:1) were more often observed on a nutritionally complementary food 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 both to 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 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; Lee et al., 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 learning scores in comparison to flies reared on more balanced diets. Highest larval performances were obtained for flies raised on P:C 1:2, which is consistent with recent estimates of *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), 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 *Drosophila* 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), *Drosophila* (Guo et al., 1996; Kawecki, 2010; Kolss and Kawecki, 2008; Shou-Zhen et al., 1997; Tully et al., 1994)], 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 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 fruits start rotting and yeast population grow, their composition 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 ripen fig fruit changes from ca. P:C 1:10 to P:C 10000:1 over the course of 27 days, with P and C concentrations varying between 10 and 10000 $g.L^{-1}$ (Matavelli et al. 2015). These nutritional modifications of food resources are likely favoured by the facts that females inoculate the fruit substrate with yeast during oviposition (Buser et al., 2014; Stamps et al., 2012), tend to lay eggs in aggregations [(Navarro and del Solar, 1975; Prokopy and Roitberg, 2001; Wertheim et al., 2005), see also experiment 2], and that multiple fly species occasionally 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 too advanced stage in the food decay process 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 in laboratory conditions where the preference for a non-nutritious substrate (media without yeast) changes depending on its distance to a nutritious substrate (media 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 definitely 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. While 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) may cause 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 allo-regulatory 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), 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 community 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 promises 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 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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Figures

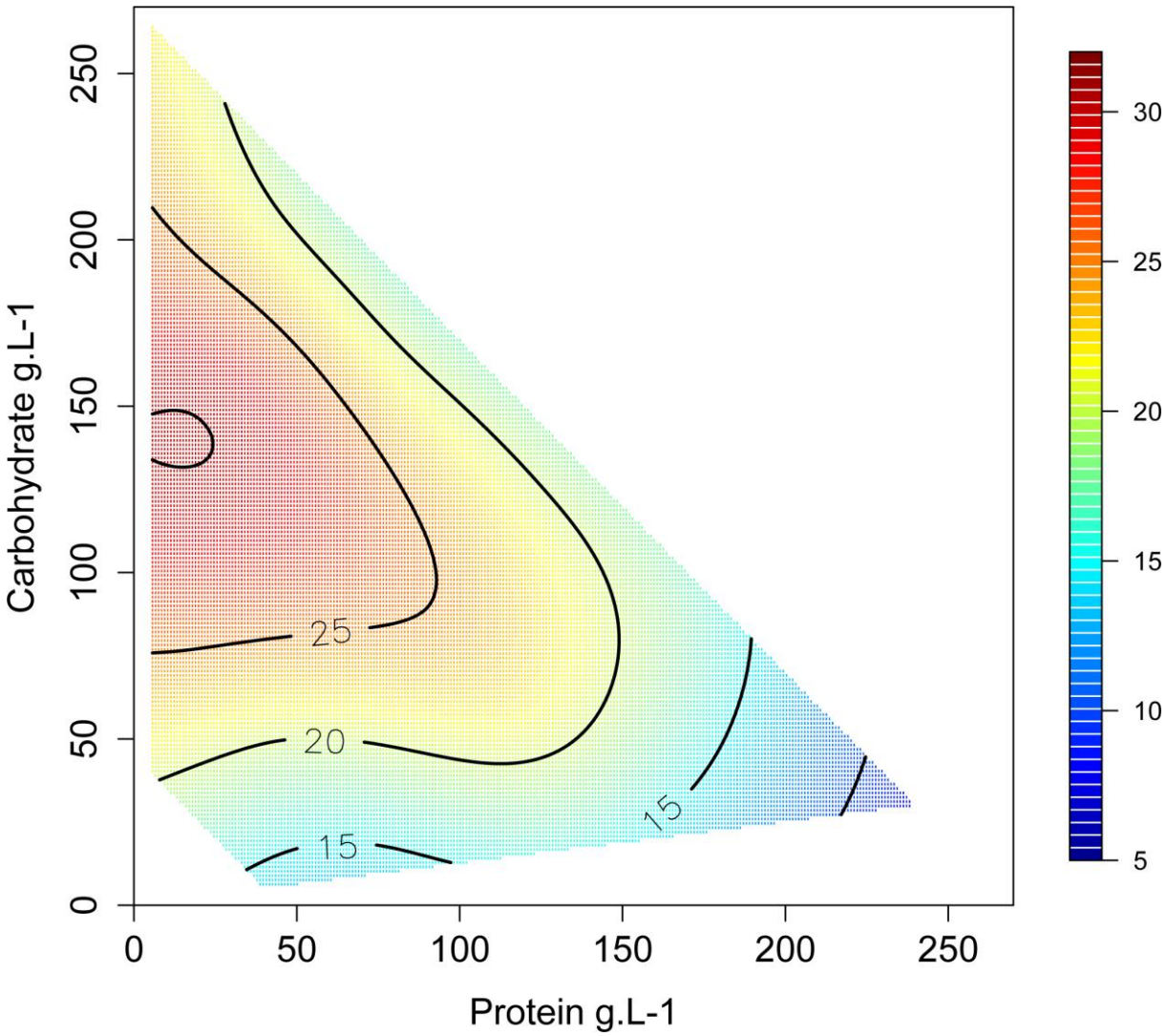


Figure 1: Egg-laying performances in no choice assays (experiment 1). Effects of nutrient balance and concentration on the number of eggs laid by individual flies confined for 24 hours to one of 34 diets (N ≥ 20 flies for each diet, Table S1). Response surfaces were generated using non-parametric thin-plate splines fitted using the *fields* package in R (Nychka et al., 2016) (see details of Lande–Arnold regression in Table S2). Dark red indicates the highest values for the number of eggs laid, with values descending to lowest values in dark blue regions.

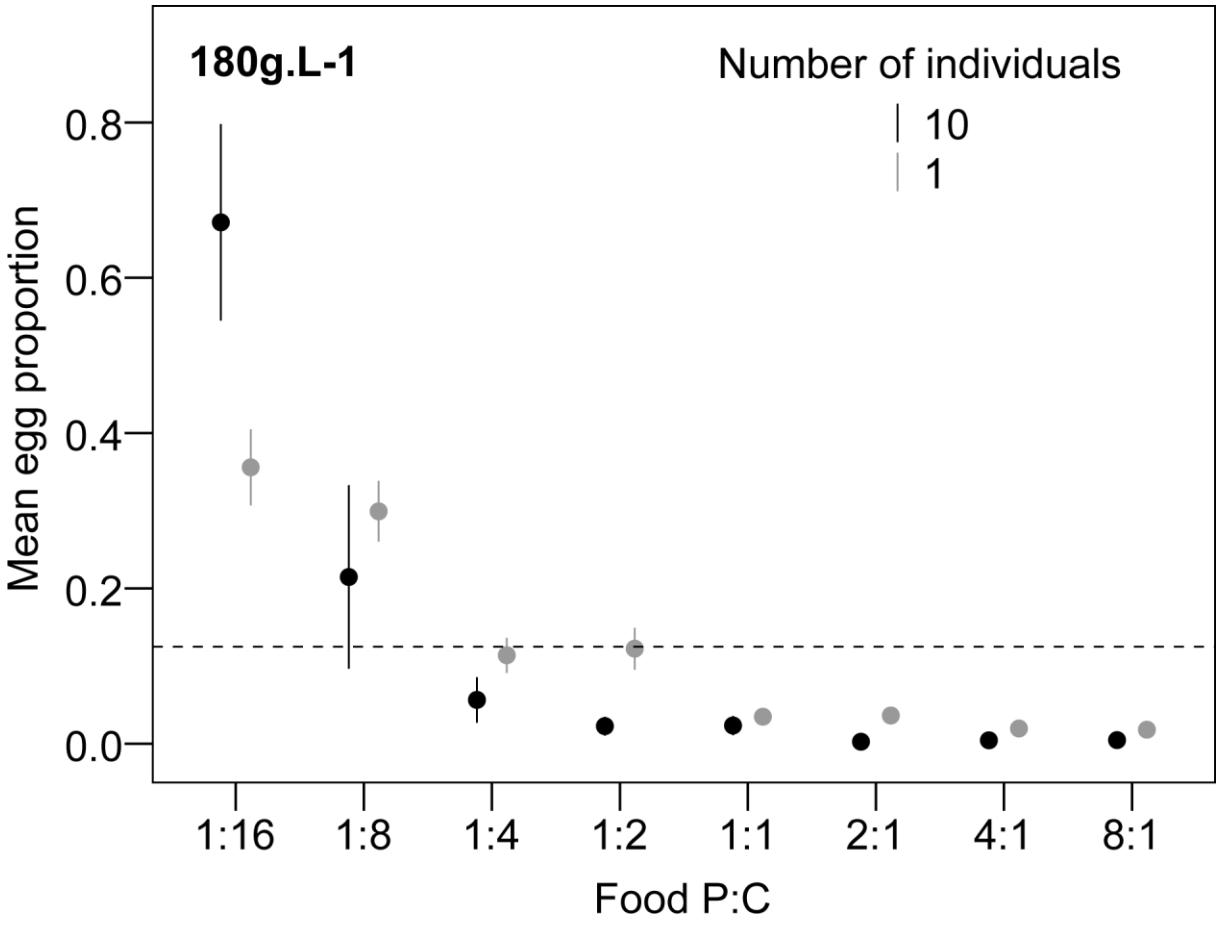


Figure 2: Egg-laying preferences in choice assays (experiment 2). Mean proportion of eggs laid on each food patch for individual (N=40 flies, Table S1) or groups (N=24 groups of 10, table S1) of flies. The dotted line indicates random choices. Error bars indicate ±95% CI.

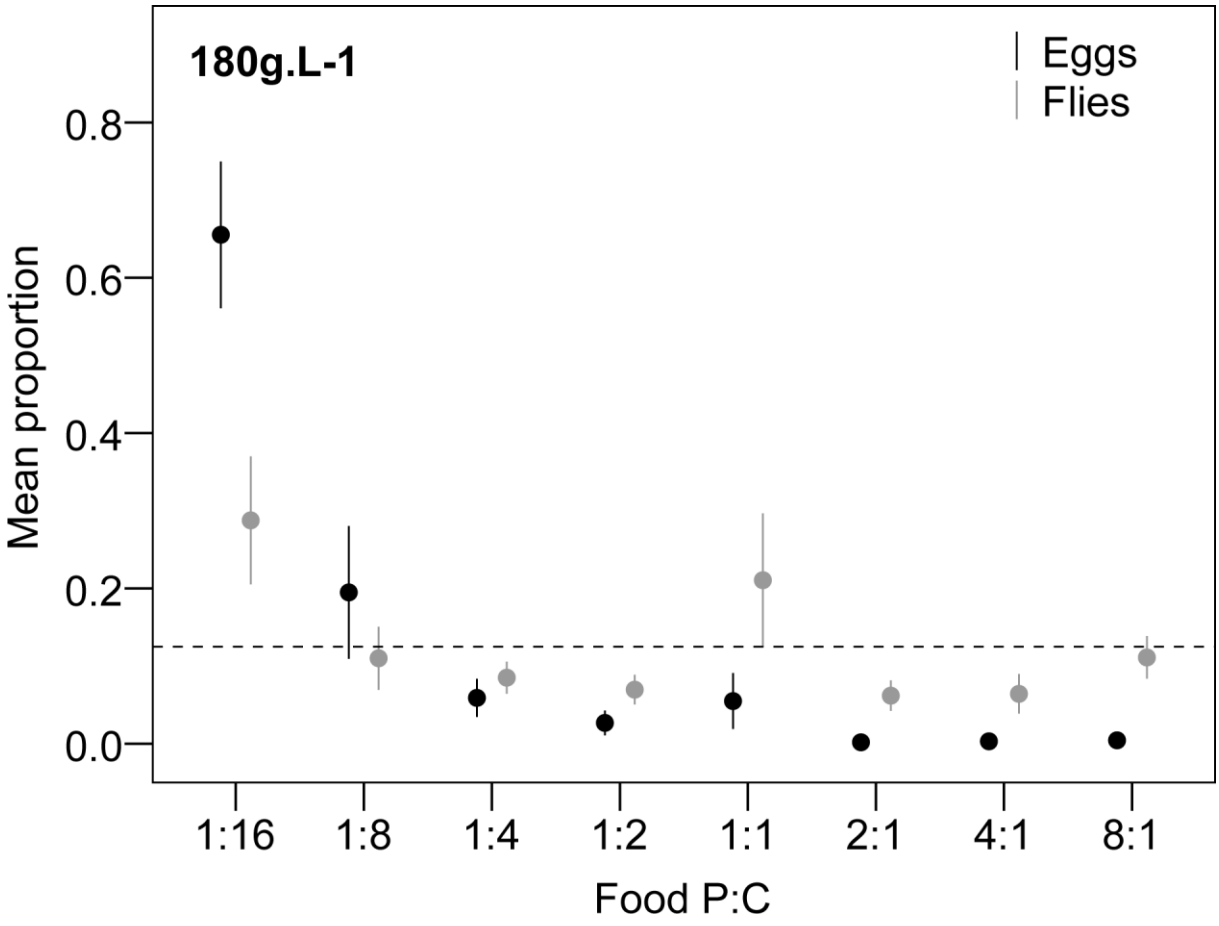


Figure 3: Interaction between feeding and egg-laying in choice assays (experiment 3). Mean proportion of flies and eggs on each diet (N=21 groups of 10 flies, Table S1). The dotted line indicates random choices. Error bars indicate ±95% CI.

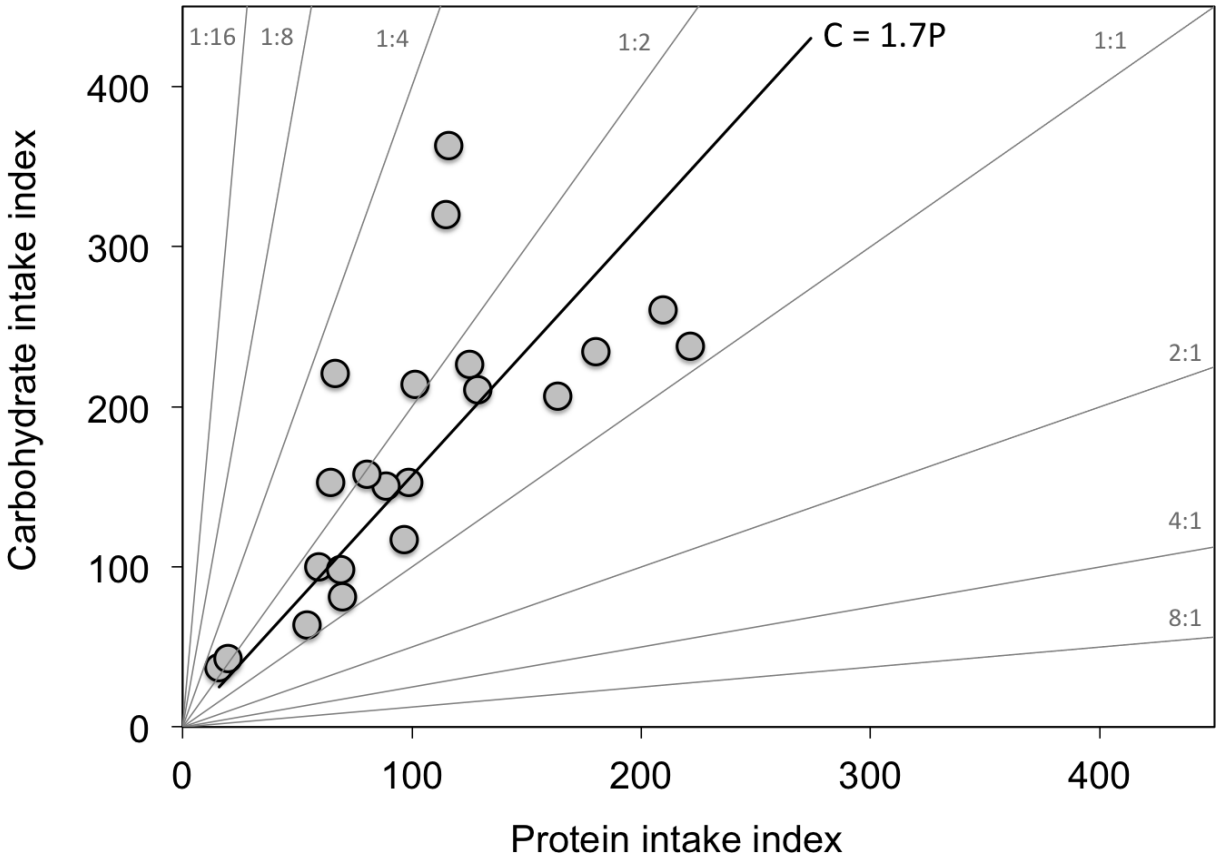


Figure 4: Nutrient intake target (experiment 3). Index of protein and carbohydrate intake computed from the time spent on each of the eight foods (N=21 groups of 10 flies, Table S1). Grey lines represent foods. Slope indicates C:P ratio of each food.

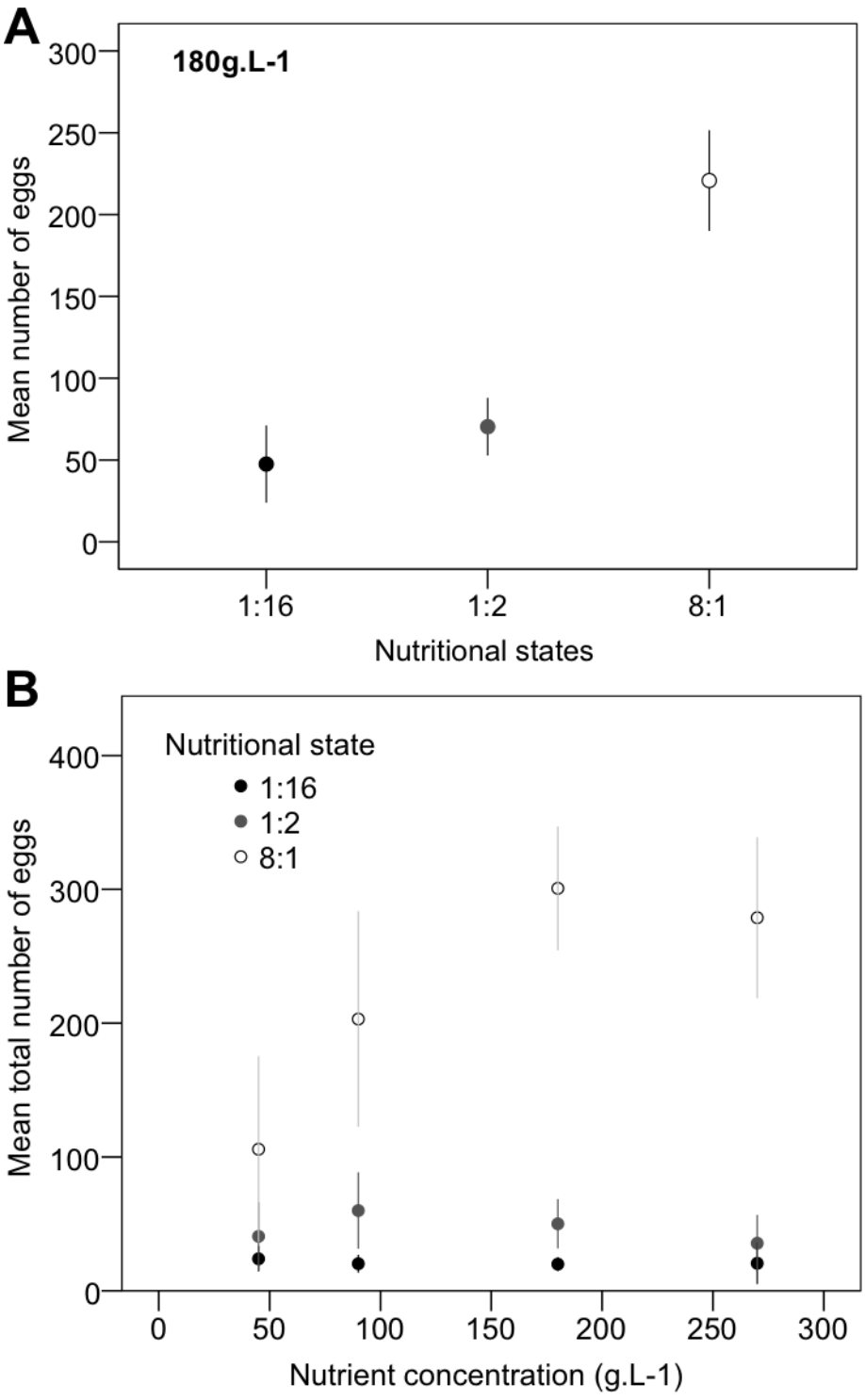


Figure 5: A. Effect of nutritional state on egg-laying performances in no choice assays (experiment 4). Mean total number of eggs laid on a standard diet according to the nutritional state of flies (N=40 flies for each nutritional states). **B. Effect of nutritional state on egg-laying preferences in choice assays (experiment 4**). 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 45g.L^{-1}, N=59 groups for 90g.L^{-1}, N=59 groups for 180g.L^{-1}, N=57 for 270g.L^{-1}). Flies were tested in groups of 10**.** Error bars indicate ±95% CI.

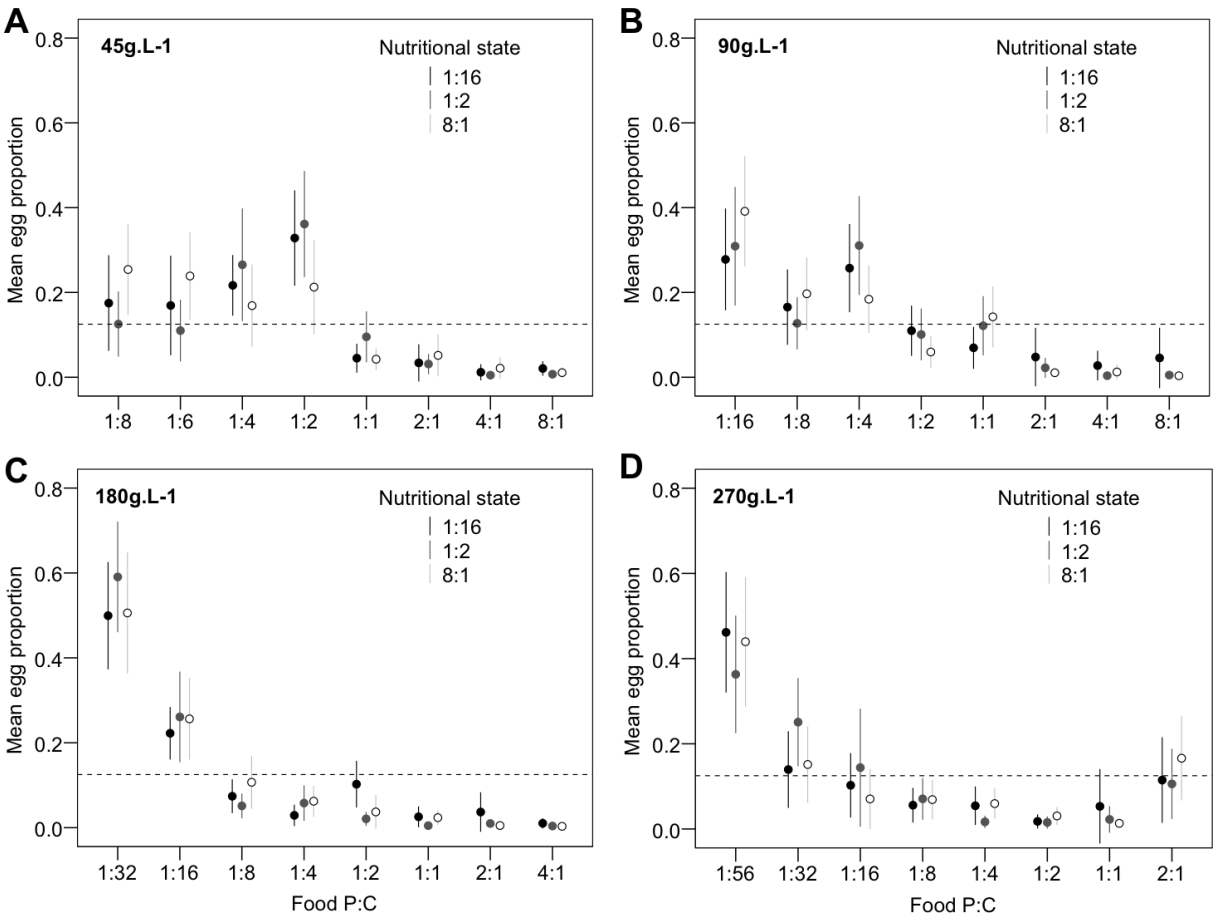


Figure 6: Effect of nutritional state on egg-laying preferences in choice assays (experiment 4). Mean proportion of eggs laid on each food according to the nutritional state of flies (N=79 groups for P:C 1:16, N=79 groups for P:C 1:2, N=77 groups for 8:1) and P+C concentration of foods (N=60 groups for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

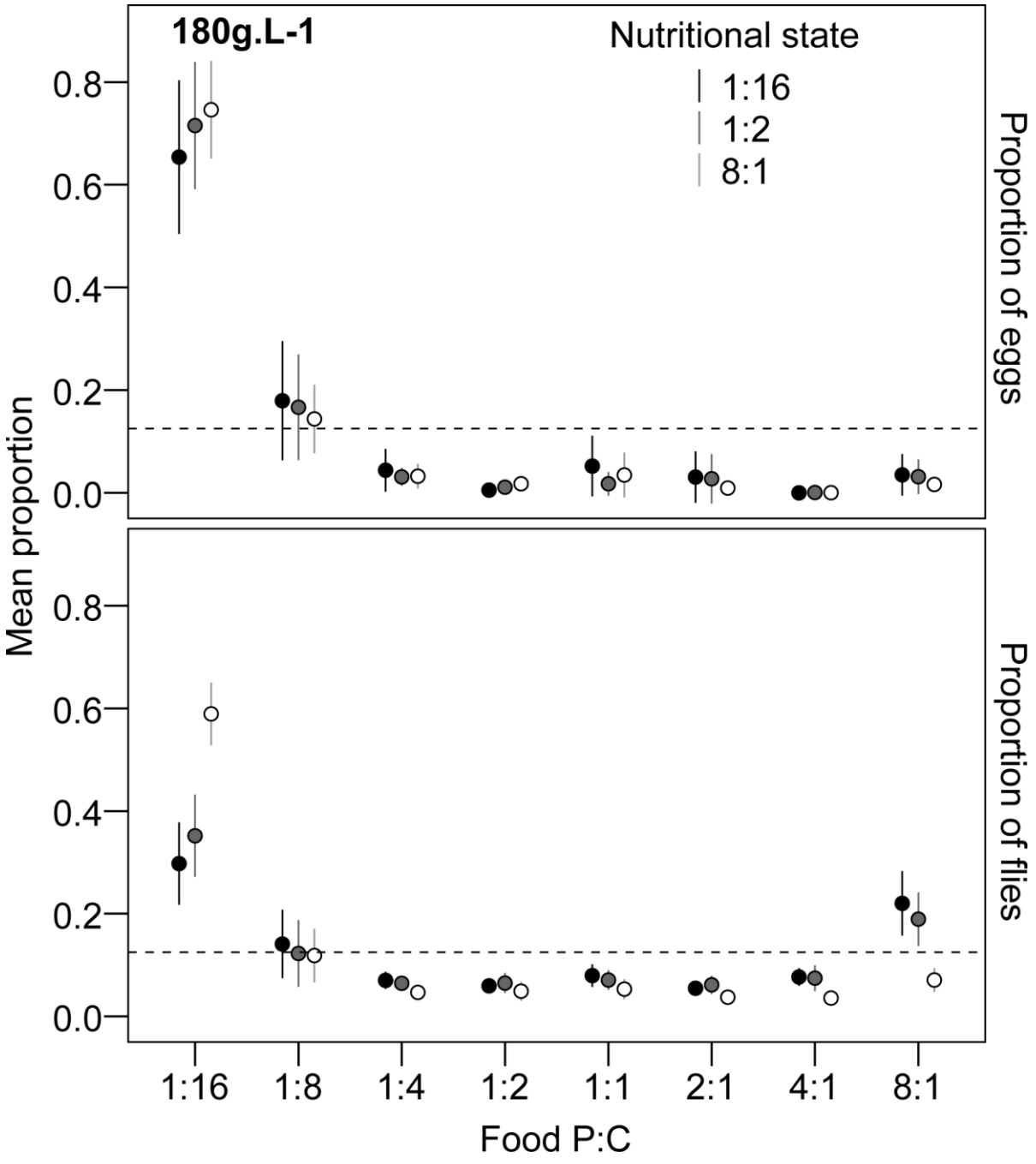


Figure 7: Effect of nutritional state on the interaction between feeding and egg-laying in choice assays (experiment 5). Mean proportion of flies and eggs on each food according to the nutritional state of flies (N=18 groups for P:C 1:16, N=17 groups for P:C 1:2, N=19 groups for P:C 8:1). The P+C concentration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

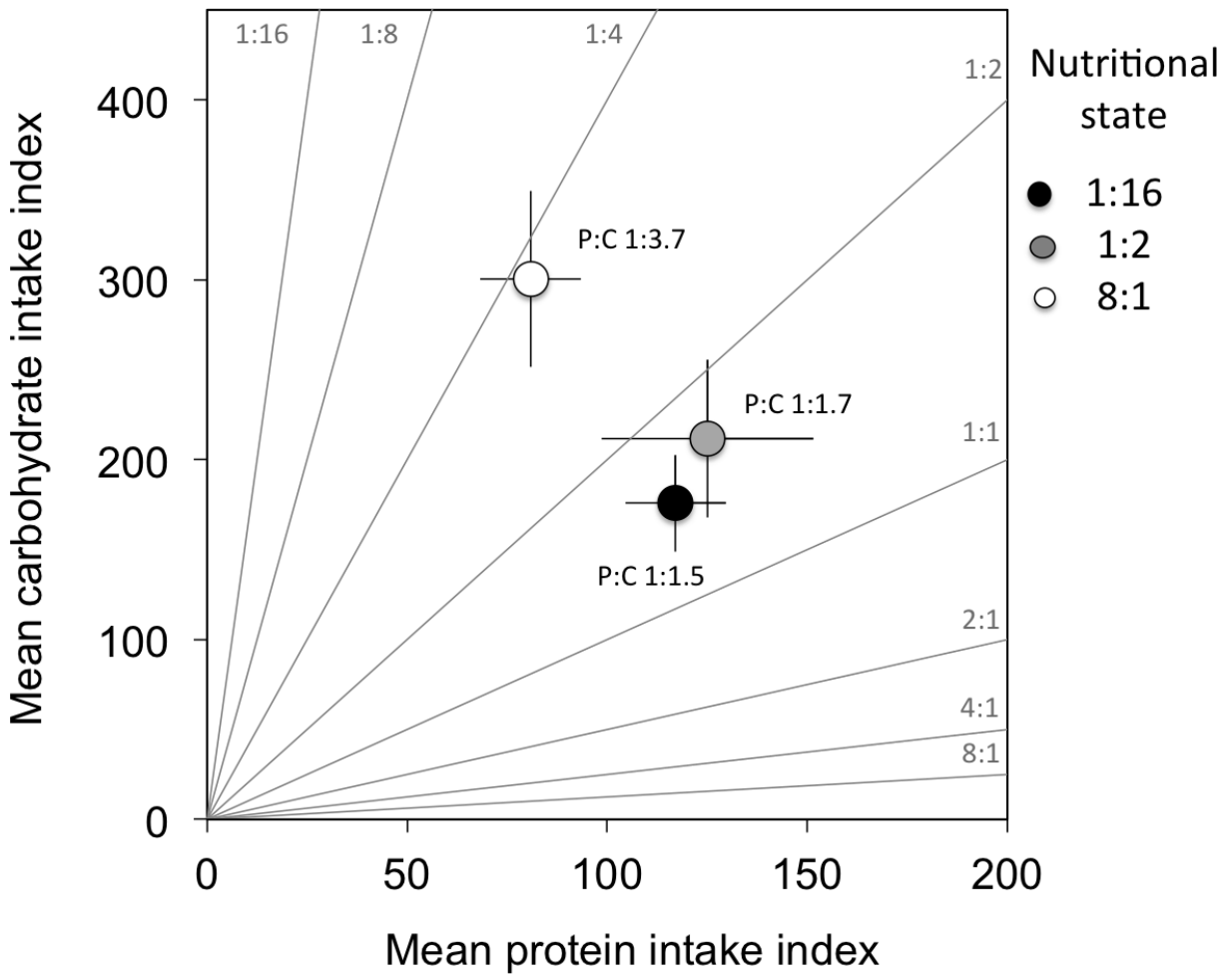


Figure 8: Effect of nutritional state on nutrient intake (experiment 5). Index of protein and carbohydrate intake computed from the time spent on foods according to the nutritional state of flies (N=18 groups for P:C 1:16, N=17 groups for P:C 1:2, N=19 groups for P:C 8:1). Flies were tested in groups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

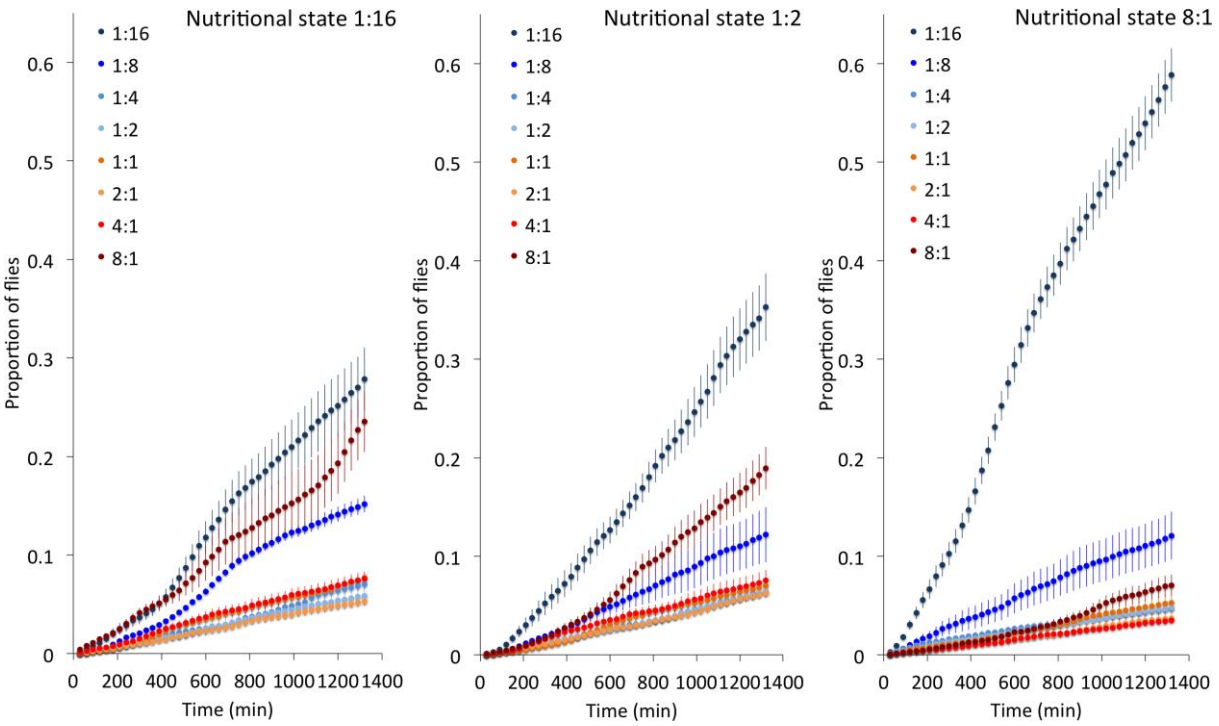


Figure 9: Effect of nutritional state on food visitation dynamics (experiment 5). Cumulated proportion of flies observed on each food (N=18 groups for P:C 1:16, N=17 groups for P:C 1:2, N=19 groups for P:C 8:1). Flies were tested in groups of 10**.** The colour code indicates variance in P:C ratios,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

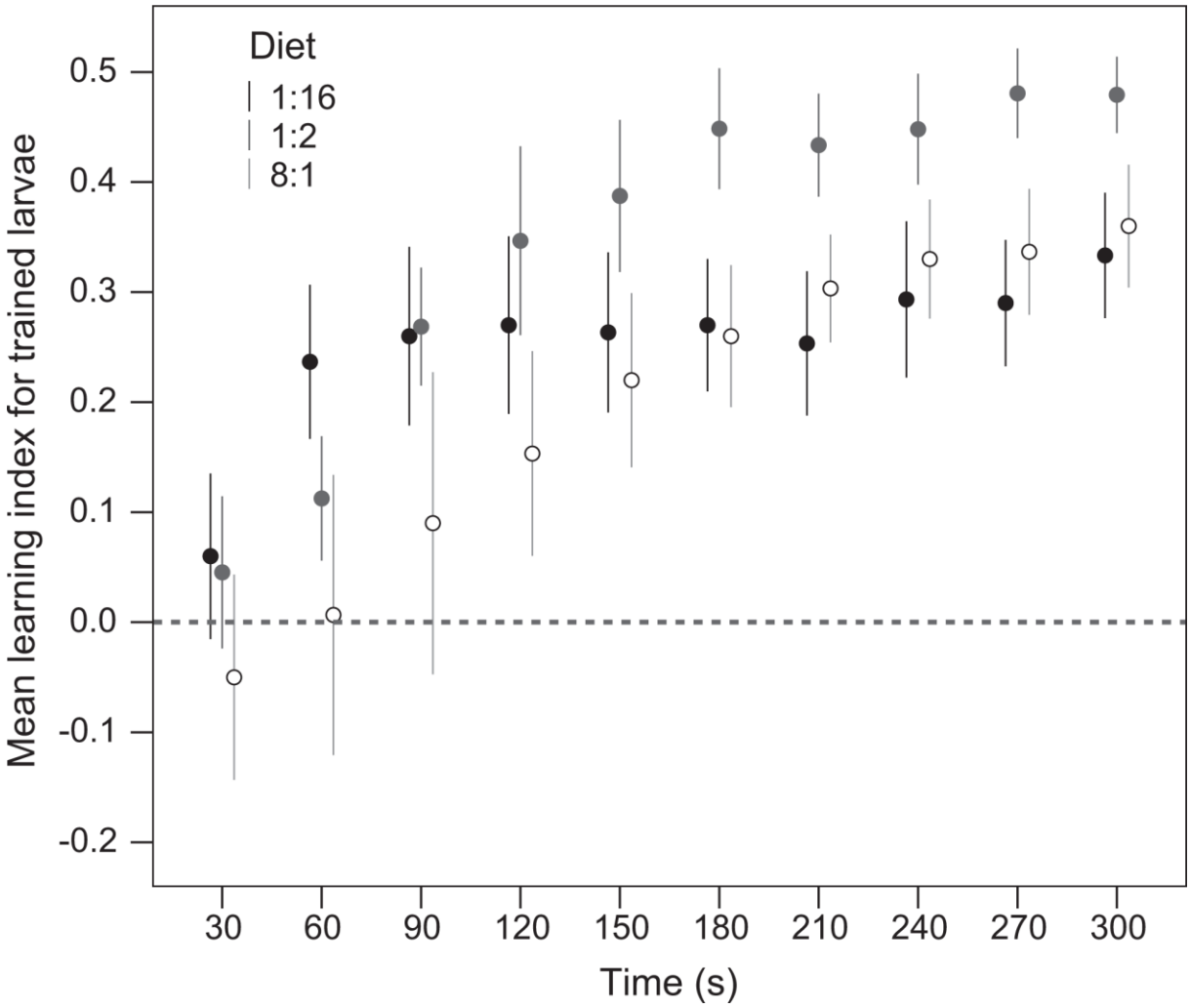
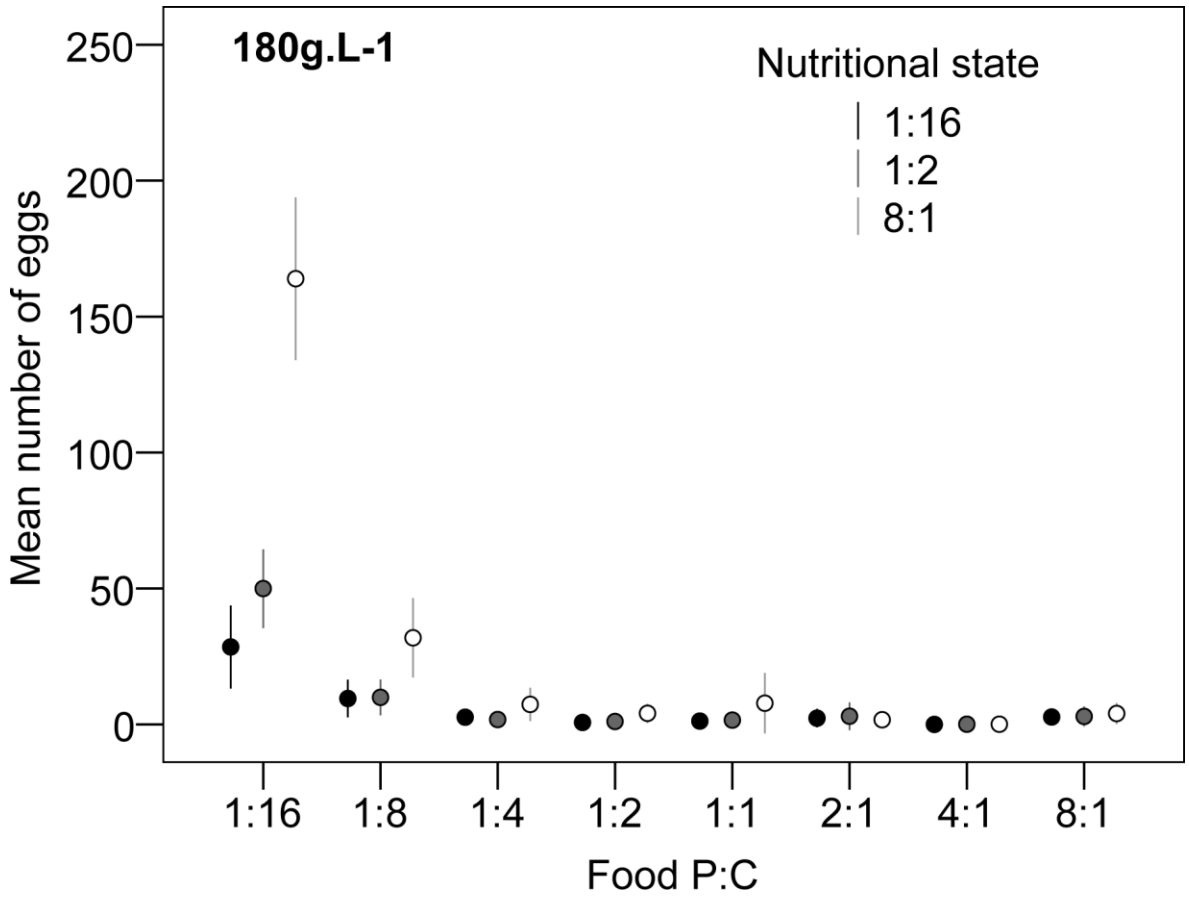


Figure 10: Effect of nutritional state on larval cognition (experiment 7). Learning index (LI) according to the nutritional state of flies (N=10 groups of 30 larvae for each nutritional state). LI ranges from -1 to 1. Positive values indicate successful associative learning. Error bars indicate ±95%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



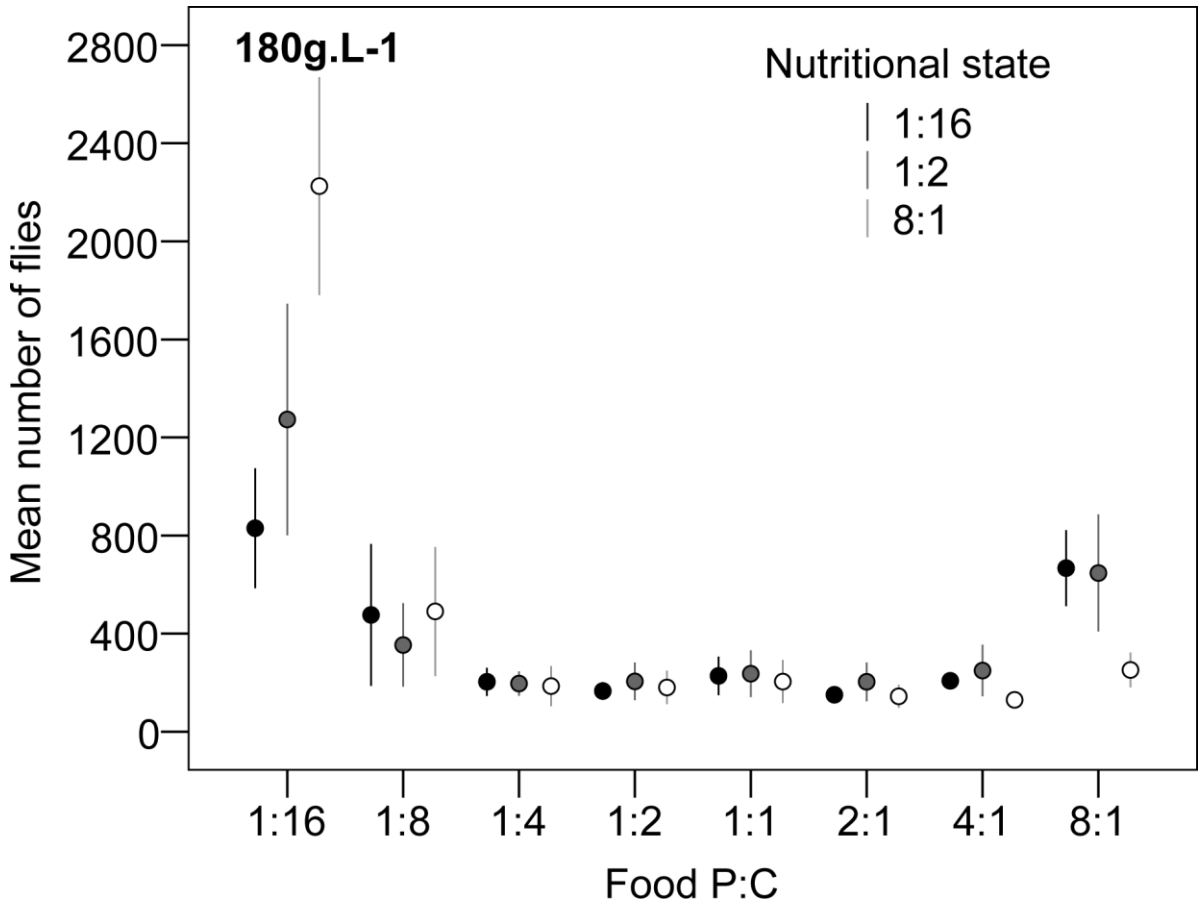


Fig. S1. Effect of nutritional state on the interaction between feeding and egg-laying in choice assays (experiment 5). Mean number of flies (A) and eggs (B) on each food according to the nutritional state of flies (N=18 groups for P:C 1:16, N=17 groups for P:C 1:2, N=19 groups for P:C 8:1). The P+C concentration was 180g.L^{-1} for food. Flies were tested in groups of 10**.** Error bars indicate ±95% CI.

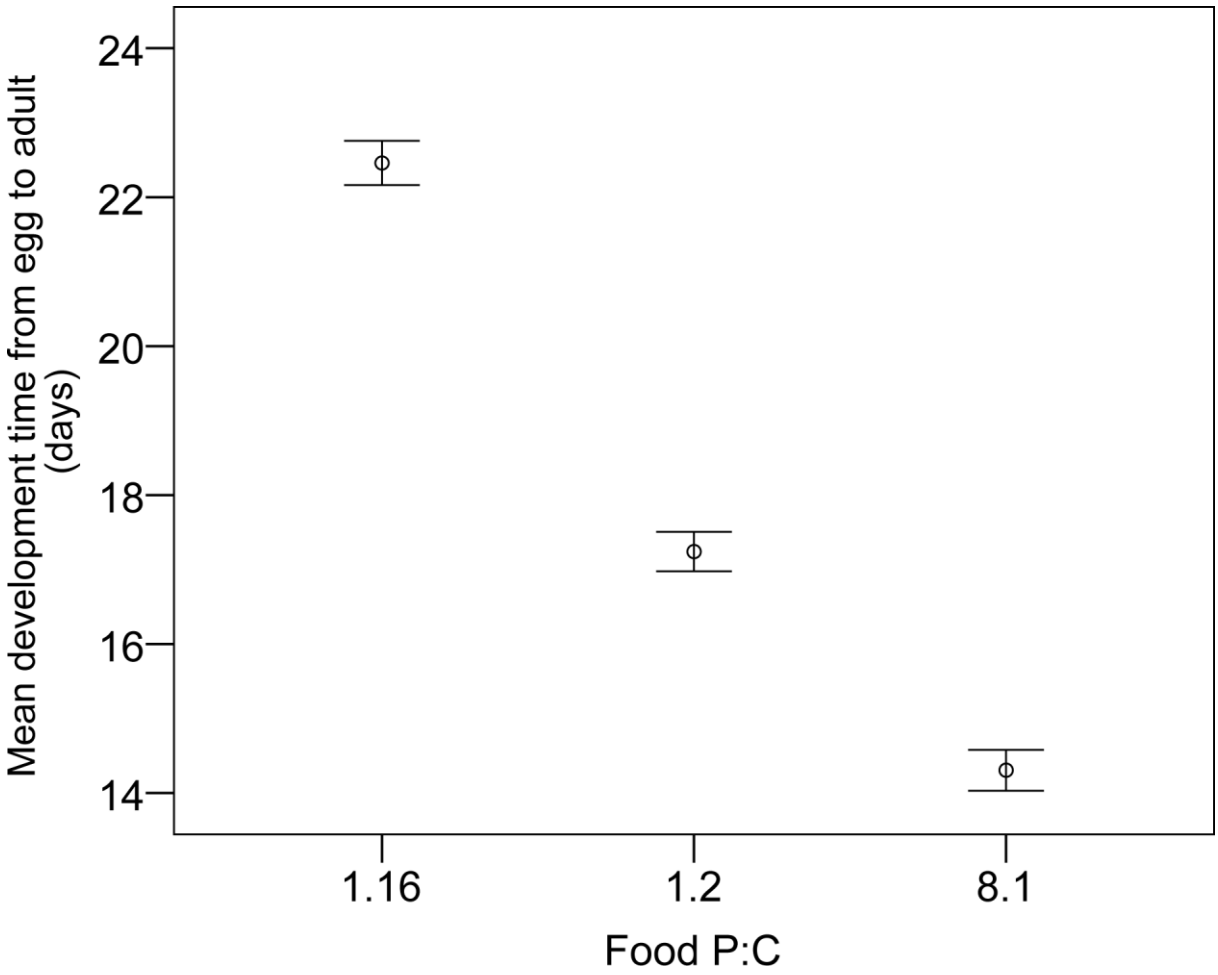


Fig. S2. Effect of breeding diet on the development time from egg to adult (experiment 6). Three protein to carbohydrate ratios (P:C) were tested. Flies were tested in groups of 5 (N=15 groups per food, N=1518 egg in total)**.** Error bars indicate ±95% CI.

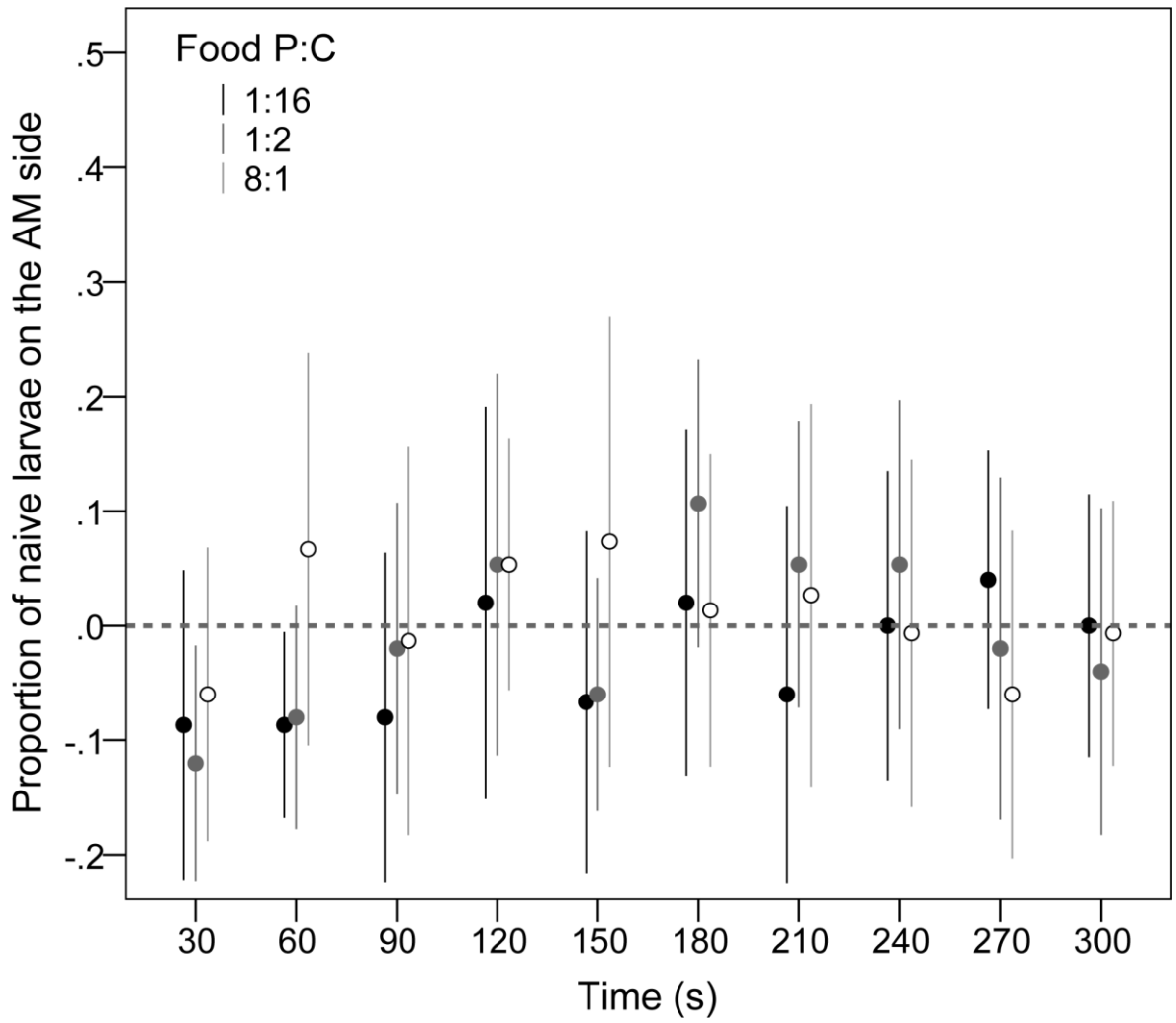


Fig. S3. Effect of nutritional state on larval odour preference (experiment 7). Proportion of larvae observed on the AM side after 3 minutes according to the nutritional state of flies (N=10 groups of 30 larvae for each nutritional state). Error bars indicate ±95% CI.

Table S1. Details of the experimental designs. P:C is the protein to carbohydrate balance in diets. P+C is the total concentration of protein and carbohydrate ($g.L^{-1}$). N is the number of replicates.

Experiment	Breeding diet	Number of flies	Type of choice	Characteristics of food patches		N	Variable Recorded
				P+C	P:C		
Exp. 1	Standard	1	no choice	45	8:1	20	Number of eggs
	Standard	1	no choice	45	4:1	20	
	Standard	1	no choice	45	1:1	20	
	Standard	1	no choice	45	1:2	20	
	Standard	1	no choice	45	1:4	20	
	Standard	1	no choice	45	1:6	20	
	Standard	1	no choice	45	1:8	20	
	Standard	1	no choice	90	8:1	20	
	Standard	1	no choice	90	4:1	20	
	Standard	1	no choice	90	2:1	20	
	Standard	1	no choice	90	1:1	20	
	Standard	1	no choice	90	1:2	20	
	Standard	1	no choice	90	1:4	20	
	Standard	1	no choice	90	1:8	20	
	Standard	1	no choice	90	1:16	20	
	Standard	1	no choice	180	8:1	28	
	Standard	1	no choice	180	4:1	37	
	Standard	1	no choice	180	2:1	37	
	Standard	1	no choice	180	1:1	37	
	Standard	1	no choice	180	1:2	37	
	Standard	1	no choice	180	1:4	37	
	Standard	1	no choice	180	1:8	37	
	Standard	1	no choice	180	1:16	37	
	Standard	1	no choice	180	1:32	20	
	Standard	1	no choice	270	8:1	20	
	Standard	1	no choice	270	4:1	20	
	Standard	1	no choice	270	2:1	20	
	Standard	1	no choice	270	1:1	20	
	Standard	1	no choice	270	1:2	20	
	Standard	1	no choice	270	1:4	20	
	Standard	1	no choice	270	1:8	20	
	Standard	1	no choice	270	1:16	20	
	Standard	1	no choice	270	1:32	20	
	Standard	1	no choice	270	1:56	20	
Exp. 2	standard	1	choice	180	8:1, 4:1, 2:1, 1:1, 1:2, 1:4, 1:8, 1:16	40	Number of eggs
	standard	10	choice	180		24	
Exp. 3	Standard	10	choice	180	8:1, 4:1, 2:1, 1:1, 1:2, 1:4, 1:8, 1:16	21	Number of eggs & Number of flies
Exp. 4	8:1	1	no choice	180	Standard diet	40	Number of eggs
	1:2	1	no choice	180		40	
	1:16	1	no choice	180		40	
	8:1	10	choice	45	8:1, 4:1, 2:1, 1:1, 1:2, 1:4, 1:6, 1:8	20	Number of eggs
	8:1	10	choice	90	8:1, 4:1, 2:1, 1:1, 1:2, 1:4, 1:8, 1:16	20	
	8:1	10	choice	180	4:1, 2:1, 1:1, 1:2, 1:4, 1:8, 1:16, 1:32	20	
	8:1	10	choice	270	2:1, 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:56	17	
	1:2	10	choice	45	8:1, 4:1, 2:1, 1:1, 1:2, 1:4, 1:6, 1:8	20	
	1:2	10	choice	90	8:1, 4:1, 2:1, 1:1, 1:2, 1:4, 1:8, 1:16	20	
	1:2	10	choice	180	4:1, 2:1, 1:1, 1:2, 1:4, 1:8, 1:16, 1:32	19	
	1:2	10	choice	270	2:1, 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:56	20	
	1:16	10	choice	45	8:1, 4:1, 2:1, 1:1, 1:2, 1:4, 1:6, 1:8	20	
	1:16	10	choice	90	8:1, 4:1, 2:1, 1:1, 1:2, 1:4, 1:8, 1:16	19	
	1:16	10	choice	180	4:1, 2:1, 1:1, 1:2, 1:4, 1:8, 1:16, 1:32	20	
	1:16	10	choice	270	2:1, 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:56	20	
Exp. 5	8:1	10	choice	180	8:1, 4:1, 2:1, 1:1, 1:2, 1:4, 1:8, 1:16	19	Number of eggs & Number of flies
	1:2	10	choice	180		17	
	1:16	10	choice	180		18	
Exp. 6	standard	5	no choice	180	8:1	15	Number of eggs & Larval development
	standard	5	no choice	180	1:2	15	
	standard	5	no choice	180	1:16	15	

Table S2. Univariate response surface regression analyses testing the relationship between the nutrient concentration (protein and carbohydrate) and the number of eggs laid (experiment 1). We used Lande–Arnold regressions to estimate parametric nonlinear response surfaces. These comprise linear and quadratic components for protein and carbohydrate intake and the cross-product of P and C. **A.** Tests of significance for the whole model. **B.** Estimated standardized coefficients. Significance: *, P < 0.05; **, P < 0.01; ***, P< 0.001.

A

	R^2	$F_{5,806}$	P
Number of eggs	0.19	38.17	<0.001

B

	Linear β (b)	Quadratic β (b)	Correlational β P × C (b)
Protein	0.454 (0.098) **	-0.368 (-0.0004) **	-0.418 (-0.0012) ***
Carbohydrate	1.800 (0.314) ***	-1.508 (-0.0010) ***	