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Effects of Early-Life Experience on Learning Ability in Fruit Flies

Zachary Durisko & Reuven Dukas

Animal Behaviour Group, Department of Psychology, Neuroscience & Behaviour, McMaster University, Hamilton, Ontario, Canada

Correspondence
Zachary Durisko, Animal Behaviour Group, Department of Psychology, Neuroscience & Behaviour, McMaster University, 1280 Main Street West, Hamilton, Ontario, L8S 4K1, Canada
E-mail: duriskzt@mcmaster.ca

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Abstract

Learning and memory require the development, modification and maintenance of brain tissue, which cost time and energy. It may be adaptive for developing animals to adjust such investments based on environmental cues indicating the future utility of learning. The optimal learning ability that maximizes fitness will vary with the degree of complexity or difficulty of the environment, and developing animals may show an adaptive plastic modification of the extent of their learning ability based on early-life cues of environmental complexity. We tested whether fruit fly larvae reared in a 'complex' environment, where they had to search, sample and choose between three foods differing in flavour and bitterness subsequently possessed greater learning abilities than larvae reared in a simple environment with only one food type. We tested learning ability both at the larval stage and in young adults. Our results suggest that, despite theoretical and intuitive appeal, these environmental factors did not affect learning ability.

Introduction

It is typically assumed that learning, defined as the ability to acquire neuronal representations of new information (Dukas 2004), will only emerge and persist in a population when its fitness benefits outweigh costs (Stephens 1991; Mery & Kawecki 2005; Dunlap & Stephens 2009). The benefits of learning can often be quite intuitive, but in general, an ability to learn allows an individual to increase its rate of resource acquisition with experience (for example, to increase the units of food or mates encountered per unit time), which typically translates into an increase of individual fitness. Researchers have documented these benefits across several ecological contexts in many laboratory experiments (Siegel & Hall 1979; Gailey et al. 1985; Dukas & Bernays 2000; Dukas & Duan 2000; Dukas 2005a) and under more natural settings (Nager & van Noordwijk 1995; Grieco et al. 2002; Dukas 2008a,b; Raine & Chittka 2008; Durisko et al. 2011).

There are also fitness costs associated with learning and memory. Learning and memory require brain tissue, which is metabolically expensive for an organism to develop and maintain (Laughlin et al. 1998; Niven & Laughlin 2008). Specifically, learning requires both the initial constitutive, or *global*, investment to develop the brain structure performing the learning and also the *induced* cost of building and maintaining each particular memory (Snell-Rood et al. 2009; Burns et al. 2011). Artificial selection experiments on fruit flies have shown that an increased learning ability is correlated with a decline in larval competitive ability and a reduction in longevity, regardless of whether an individual utilizes its learning ability, suggesting a cost of the initial investment in the ability to learn (Mery & Kawecki 2003; Burger et al. 2008). Furthermore, the act of forming a long-term memory has itself been associated with a reduction in lifespan and fecundity in fruit flies (Mery & Kawecki 2004, 2005). Snell-Rood and colleagues have shown that better-learning cabbage white butterflies (*Pieris rapae*) have fewer eggs and also that the learning process itself can reduce fecundity (Snell-Rood et al. 2011). Finally, all learners begin life inexperienced and typically exhibit an initial phase of poor performance, during which the time spent learning instead of acting may constitute an opportunity cost (Stephens 1991; Dunlap & Stephens 2009; Eliassen et al. 2009).

The ecology and neurodevelopment of each particular animal determine the costs and benefits of learning, and the balance of these selective pressures

dictates the optimal degree of learning for each environment. Learning is more beneficial in some environments than others (Stephens 1991; Dunlap & Stephens 2009; Eliassen et al. 2009), and therefore, each environment may have a different optimal degree of learning ability. A given learning ability may not be sufficient to succeed in environments that are more complex, but the same ability may be an excessive waste of time and energy in simpler environments. For animals that experience variation in environmental complexity, developing an appropriate or optimal degree of learning ability is a challenge with important fitness consequences. One way that animals may evolve to cope with such environmental variation is with adaptive developmental plasticity (Pigliucci 2001; Dukas 2004; Snell-Rood et al. 2010a). Avoiding the costs of learning whenever possible would provide an adaptive advantage. If an animal is able to assess the future value of learning, it may be able to adaptively modify the amount of time and energy invested in learning ability (Snell-Rood et al. 2009). For example, an animal experiencing cues that its future environment is likely to be very simple should adaptively reduce the energy and time devoted to developing brain tissue associated with learning. In many species, brains develop throughout early life and have the potential to be highly plastic.

We sought to document adaptive plasticity of learning ability in fruit flies (*D. melanogaster*). Female fruit flies seek out appropriate food sources in their local environment and lay eggs directly onto the surface of the food. Larvae spend much of their time eating, and we hypothesized that their foraging experience may be a relevant cue of the future utility of learning. Neurodevelopment continues throughout the larval stage, including neurogenesis in the mushroom bodies, brain structures critical for learning (Ito & Hotta 1992; De Belle & Heisenberg 1994; Tettamanti et al. 1997; Fahrbach 2006; Campbell & Turner 2010), and although the brain undergoes substantial reorganization during metamorphosis (Armstrong et al. 1998), increased neurogenesis due to environmental complexity could lead to improved learning abilities in later life. We imagined a scenario where environmental cues that indicate the future utility of learning would increase an animal's investment in mushroom body neurogenesis, which has been associated with improved learning ability (Snell-Rood et al. 2009). We hypothesized that the complexity of the larval food environment would be an ecologically relevant cue for the plasticity of learning ability and that fruit flies have evolved to adjust their investment in learning ability such that flies experiencing a challenging early-life environment would subsequently show greater learning abilities than those from a simple environment. Specifically, we predicted that early-life larval exposure to multiple food types of varying flavour and bitterness would be experienced as more complex and result in an increased learning ability compared to early-life larval exposure to a single food type. We conducted two experiments, testing the appetitive learning ability of both larval and adult life stages after exposure to either a *complex* or *simple* early-life environment.

Methods

General Methods

We maintained two population cages of several hundred *Drosophila melanogaster Canton-S* on abundant standard food, one litre of which contained 75 g cornmeal, 20 g agar, 60 g dextrose, 30 g sucrose, 32 g yeast and 2 g methyl paraben. We kept flies at 25°C, 60% relative humidity, and on a 12:12 light/dark cycle with lights on at 11 p.m. This irregular light cycle allowed peak egg laying to occur midday so that we could collect experimental eggs within a very small window of time (1.5 h). We collected eggs on 90-mm petri dishes filled with 10 ml of standard food and covered with 0.7 ml of live-yeast suspension to stimulate egg laying (30 g/l of warm water; Sarin & Dukas 2009). We kept egg density low (<200) to optimize larval development. Immediately following egg laying, we moved egg dishes to an incubation chamber maintained at 25°C, high humidity and total darkness. We conducted all further manipulations under red light, which fruit flies cannot see (Bertholf 1932), to minimize disturbance. First instar larvae fed on abundant standard food for 24 h. On day 1 of the experiment, we gently rinsed larvae from the food medium with water and moved 50 randomly selected early-second-instar larvae next to the food in each of the treatment dishes (see below).

For Experiment 1, where we tested larval learning ability, it was especially critical to control larval age and stage of development, so we added two additional steps to minimize variation. First, prior to experimental egg collection, we allowed females to lay eggs for 1 h on dishes that we discarded. This ensured that females did not lay partially developed embryos during experimental egg collection. Second, at 7 a.m. on the day following egg laying, two hours prior to the expected start of hatching (expected 22 h following egg laying), we manually removed any early-hatching larvae.

Early-experience treatments

To create environments that we predicted would be experienced as *simple* and *complex*, we modified successful insect protocols, which varied the number and qualities of alternative food sources (Dukas & Real 1993a,b; Bernays 1998; Bernays & Funk 1999; Gegear & Laverty 2005). Each treatment received identical nutrients but different arrangements of food in 60-mm petri dishes containing a thin base of agar and methyl paraben (2 g/l) (Fig. 1). We flavoured food with 20 ml/l of commercially available flavour extract: anise, lemon, or mint. *Simple* treatments consisted of a single food flavour and quality in a single patch containing 0.3 ml food. *Complex* treatments consisted of all three flavours, one of which had added quinine (2.5 g/l of quinine hydrochloride, Sigma), which tastes bitter and is aversive to larvae (Dukas 1999), arranged into three separate patches each containing 0.1 ml food located 1 cm apart. On the mornings of days 2 and 3, we added new food to the dishes, directly to the large patch in *simple* treatments, and by creating new small patches in *complex* treatments for a total of nine small food patches (Fig. 1). Note that all dishes received identical quantities of food. We added food daily instead of providing food in abundance at the outset so that the larvae from the *complex* condition depleted the small patches, forcing them to search, sample and choose whether to feed on the other flavours, likely experiencing the multiple flavours and food qualities in their environment. Larvae typically consumed the entirety of a food patch and thus on subsequent days were forced to either try a new flavour or search for the previous flavour, both of which may be cues of environmental complexity. In the *simple* dishes, larvae were free to crawl and dig in one abundant food patch. We accounted for all flavour and quinine combinations with six treatments, three *simple* and three *complex*, respectively: (1) anise, *A*; (2) lemon, *L*; (3) mint, *M*; (4) anise, lemon and mint-quinine, *ALMq*; (5) anise, lemon-quinine and mint, *ALqM*; (6) anise-quinine, lemon and mint, *AqLM*. For the test of larval learning ability, larvae experienced these treatments for 72 h (from 24 to 96 h old) including almost all of their second instar and approximately half of their third instar stage. For the test of adult learning ability, we left larvae in these dishes until eclosion.

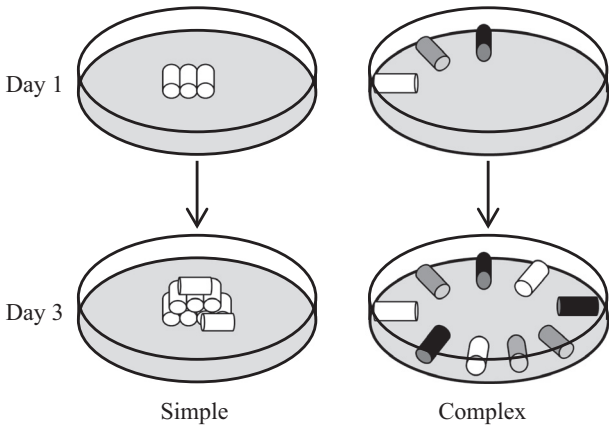

Fig. 1: We gave larvae identical nutrients in either a *simple* or a *complex* foraging environment. In our simple treatments (left), we gave larvae one flavour and quality of food in a single patch. In our complex treatments (right), we gave larvae three flavours of food, one of which contained added quinine, which tastes bitter, arranged in separate patches. We added food to all dishes each day, into one large patch for the simple treatments and for a total of nine small patches in complex treatments. Flavours used were lemon, anise and mint.

Experiment 1 – Larval Learning Ability

On the morning of day 4, at 96 h old, we assessed the learning ability of the larvae. We chose this time so that larvae had extensive experience with their foraging treatment while remaining well within the feeding stage of their third instar. We first collected the larvae from the dishes with a soft paintbrush and rinsed them in small droplets of water. We collected all living larvae and were therefore able to calculate the mortality rate for each dish. The overall mean mortality rate was very low, 0.049 ± 0.005 (x ± SE) larvae per dish, corresponding to 47.5 ± 0.2 living individuals per dish. Mortality did not differ between simple and complex dishes ($F_{1,54}$ = 2.644, p = 0.110) or flavours (nested within complexity; $F_{4,54}$ = 0.161, p = 0.957).

Pupation typically occurs more than 24 h after our chosen time of testing. Approximately 8 h prior to the formation of the puparium, or prepupa, third instar larvae cease feeding and enter the wandering stage (Roberts & Standen 1998). Wandering stage larvae would largely ignore the unconditioned food stimulus in our test of learning ability and would therefore have spuriously lower learning scores. To ensure that we tested larvae prior to wandering, we tested all larvae between 9 a.m. and 12 p.m., and after each test, we placed the larvae onto plain agar dishes where they could pupate. We reasoned that any larvae in their wandering stage during testing would begin pupation within 8 h without additional food. We counted pupae at 8 p.m. from 54 dishes (missing six

due to experimental error) and again at 9 a.m. the following day to assess differences between treatments. Very few larvae entered pupation in 8 h following the test (1.1 ± 0.4 individuals per dish, or 2.4 ± 0.9% of larvae per dish). There was no difference in the proportion of larvae entering pupation at 8 p.m. between the simple and complex dishes, (respectively, 2.7 ± 1.7% and 2.1 ± 0.7%; $F_{1,48} = 0.2$, p = 0.67) or among the different flavours ($F_{4,48} = 0.9$, p = 0.50). At 9 a.m. the following day, 21–24 h after testing, there were 10.6 ± 1.4 pupae per dish, or 23.8 ± 3.5%, and there continued to be no difference between simple and complex dishes ($F_{1,54} = 1.9$, p = 0.17) or flavours ($F_{4,54} = 1.3$, p = 0.29).

Training and test of larval learning ability

The learning test consisted of a group reciprocal conditioning assay with one of two novel odours paired with fructose-flavoured agar (2 M) as a rewarding stimulus (the 'rewarded odour') and the other paired with plain agar (similar to Aceves-Piña & Quinn 1979; Dukas 1999; Scherer et al. 2003; Neuser et al. 2005). We conducted all training and tests under a fume hood. We balanced the odour paired with fructose across replicates to control for any innate odour preference. We rotated the order in which we tested the six treatments and balanced which training odour/food pair we presented first to control for any order effects. We used the chemical odorants 1-butanol (BUT; Fisher) and propyl acetate (PA; Sigma), and we diluted the latter 1:300 in paraffin oil (a concentration at which naïve larvae preferred the two odours approximately equally in preliminary trials). Both odours are strongly attractive to larvae and have been used in similar larval learning tests (Kaun et al. 2007). For each training session, we filled a small plastic cup (polypropylene NMR tube caps, Sigma) with 10 μl of odourant and placed it onto the centre of a 60-mm petri dish filled either with plain agar or with fructose-flavoured agar. We placed larvae directly into these dishes en masse with a paintbrush. The petri dish lids remained on the dishes during training so that odours vapours collected in the dish, but we perforated each lid with 16 1-mm holes around the perimeter to improve aeration (similar to Neuser et al. 2005). We moved larvae manually between training sessions, alternating between each odour/food pairing. Each training session lasted 5 min, and between each session, we gave the larvae 1-min breaks in a droplet of clean water. This served to rinse any agar or sugar from the previous training session and such breaks improve learning scores (Scherer et al. 2003). Each group of larvae received six training sessions, three of each odour/food pair, lasting a combined total of 35 min.

Immediately following training, we transferred larvae to a clean water droplet for 1 min before giving an odour preference test. We conducted tests in 90-mm-diameter petri dishes containing a thin layer of agar. We placed the larvae along the midline, equidistant from two odour cups filled with 10 μl of the respective odours at opposite ends of the dish. Each odour cup sat atop a 1-cm disc of fructose-flavoured agar, which served to reward the larvae so that they did not crawl back across the midline in continued search for food after making a choice. We perforated the dish lid with holes along the midline to draw the odours towards the centre and to prevent the odours from mixing as much as possible. We spun each dish prior to testing to randomize the side of odour presentation and to ensure that the experimenter was blind to odour identity. Larvae crawled freely for a 1-min choice phase, after which we immediately counted the number of larvae on each side of the dish. Larvae within 1 cm of the midline were omitted from analysis. We regarded larvae on either side of the dish as having chosen the corresponding odour and calculated the proportion of larvae choosing the odour previously paired with fructose. Thus, a proportion of 0.5 indicated random choice and 1 indicated perfect learning.

For a single replicate, we tested six dishes, one per flavour treatment dish and three for each complexity. We repeated the experiment for 10 replicates (N = 60 total dishes: 10 of each flavour or flavours, 30 of each complexity).

Experiment 2 – Adult Learning Ability

Fly population cages, egg laying and treatment environments were identical to Experiment 1. Flies underwent pupation and eclosion within the treatment dishes. As eclosion typically takes place across a few days, we monitored the dishes daily and used any newly eclosing flies from several replicates and days for each test. We gently aspirated the flies into vials of standard food at a density no greater than 20 flies per vial. On the evening before testing, we transferred flies to vials containing plain agar and left them overnight for 16–18 h of starvation. All flies were less than 42 h old at the time of testing. We collected 38.7 ± 2.0 flies per test, and this did not differ between simple or complex tests ($F_{1,42} = 2.1$, p = 0.150) or flavours ($F_{4,42} = 1.4$, p = 0.243).

Adult training and test

We tested adult learning ability by exposing flies to two novel odours, 3-octanol (OCT, Fluka) and 4-methylcyclohexanol (MCH, Fluka), with one odour paired with a dried filter paper that had previously been soaked in 2 M sucrose solution (the 'rewarded' odour) and the other odour paired with plain filter paper, followed by a test of odour preference (test adapted from Tully & Quinn 1985; Schwaerzel et al. 2003; Thum et al. 2007). Prior to training, we exposed sugar filter papers to 20 flies for 5–10 min to scent the filter paper and promote the learning of experimental flies (Connolly & Tully 1998). We aspirated flies from the six treatments into six randomly numbered empty vials and tested in random order, blind to treatment. The testing apparatus consisted of a Plexiglas elevator chamber that moved the flies from a training tube, which was lined with the filter paper, to a point between two choice tubes (similar to Tully & Quinn 1985). Odour concentrations were adjusted by dilution in heavy mineral oil beforehand to 1:50 MCH and 1:250 OCT, concentrations that naïve flies preferred approximately equally in preliminary trials. A vacuum pump drew air through small, 50-ml flasks, bubbling through the odorant-oil mixtures and then through the training or choice tubes of the experimental apparatus and out of the room. We monitored and controlled odour flow to 14 ml/s per tube. The vacuum pump remained on for the entirety of training and testing so that the flies habituated to the noise and so that clean room air could clear the previous odour between trainings. We aspirated flies into the apparatus and let them rest for 90 s in the elevator chamber. We exposed flies to the first odour/filter-paper pairing for 60 s, gently shook them back into the elevator chamber, gave 30-s rest and then exposed them to the second odour/filter-paper pairing for 60 s. Following this, we gently shook the flies into the elevator, gave 90-s rest and finally moved them into a T-maze choice point at the convergence of the two odour streams for a 60-s choice phase where they could enter the tube containing their preferred odour. We conducted all training and tests under red light with the final rest and choice phases conducted in complete darkness to eliminate any phototactic behaviour. We gave flies one training cycle and test only, the entirety of which took less than 6 min: (1) 60 s of OCT; (2) 30-s rest; (3) 60 s of MCH; (4) 90-s rest; (5) 60-s choice between the odours. Following the choice phase, we anesthetized flies with CO_2 and counted the proportion of flies choosing each odour. Flies remaining in the centre were omitted from analysis. We balanced the odour paired with sugar and the side of odour presentation during the choice phase across replicates. We tested eight replicates of the six treatments for a total of 48 tests, 24 of each complexity.

Data Analysis

All proportion data were arcsine square root transformed prior to statistical tests (Sokal & Rohlf 1995, p. 419) and met ANOVA assumptions after transformation. We assessed the learning abilities of larvae and adults with an ANOVA on the proportion of larvae choosing PA or MCH respectively, chosen arbitrarily. Significant learning in the larval case was indicated by increased preference for PA, while PA was paired with fructose and decreased preference for PA while BUT was paired with fructose. Similarly, in the adult case, learning was indicated by increased preference for MCH when MCH was paired with sucrose and a decreased preference for MCH while OCT was paired with sucrose. We assessed differences in learning abilities with an ANOVA of the proportion of larvae or adults choosing the previously rewarded odour. In all analyses, *complexity* was included as a fixed factor and *flavour(s)* as a factor nested within complexity. For the larvae, we included *rewarded odour*, *odour presented first during training*, and their interaction in the model. For the adults, we included *rewarded odour* and *side of odour presentation during testing*.

For clarity and simplicity, we leave our data as the proportion of larvae or flies choosing the rewarded odour, rather than compute the *Learning Index* used by others (e.g. Neuser et al. 2005). Such indices are typically computed as the difference in the proportion of larvae choosing the rewarded odour across two reciprocally trained groups to account for underlying odour preference, then dividing by 2, with results ranging from −1 to 1, with 0 indicating random choice. In contrast, our proportions range from 0 to 1, with 0.5 indicating random choice, and we directly control and assess underlying odour preference in our statistical models, including interaction effects for conditions with different training odours.

Results

Larval Learning Ability

Larvae from complex dishes did not exhibit greater learning than larvae from simple dishes (proportion choosing the rewarded odour, respectively:

0.590 ± 0.029 and 0.572 ± 0.024, N = 60; $F_{1,51}$ = 0.4, p = 0.55; Fig. 2a). Overall, the pairing of an odour with fructose had a significant effect on subsequent odour preference ($F_{1,51}$ = 25.3, p < 0.001), indicating significant learning, with an overall x proportion of 0.581 ± 0.019 choosing the rewarded odour. There was a slight odour preference for PA, despite our attempt to balance odour preference with preliminary tests, as indicated by a significant effect of *rewarded odour* on the proportion of larvae choosing the rewarded odour ($F_{1,51}$ = 13.7, p = 0.001). Nevertheless, pairing an odour with fructose increased preference for this odour (proportion choosing PA when PA+, 0.642 ± 0.026, vs. BUT+, 0.480 ± 0.021). There were no significant effects of *flavour(s)* (nested within complexity), *complexity*, *odour presented first during training*, or the interaction between odour order and the odour paired with fructose, on either the proportion of larvae choosing PA or the proportion of larvae choosing the rewarded odour (all F < 1.9, all p > 0.178). *Post hoc* ANOVAs within each complexity revealed no significant differences between flavours (p > 0.350). Similar analysis showed that the proportion of larvae in the centre of the dish (not making a choice) did not differ due to *flavour(s)* ($F_{4,51}$ = 0.5, p = 0.7) or *complexity* ($F_{1,51}$ = 0.2, p = 0.6), with an overall x of 0.381 ± 0.015.

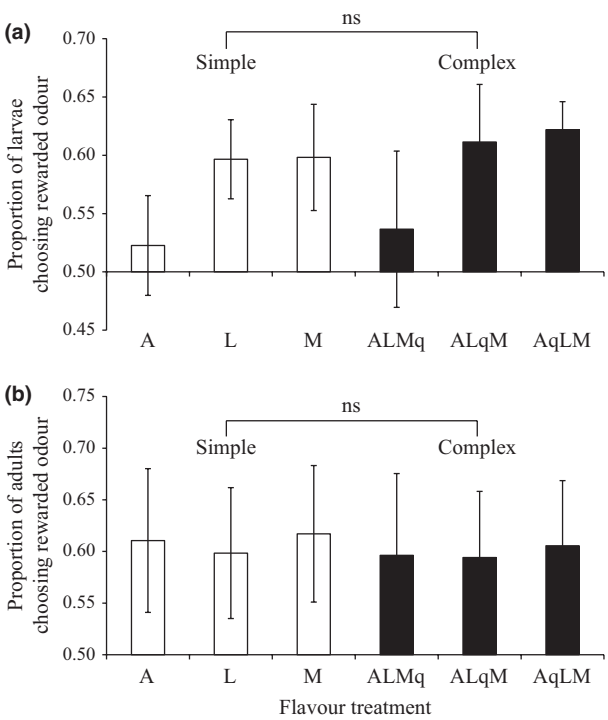

Fig. 2: Proportion of individuals choosing the rewarded odour (x ± SE) after rearing in our *simple* or *complex* arrangement of foods. Simple treatments (white bars) received one patch of one flavour of food: anise (A), lemon (L) or mint (M). Larvae from complex treatments (black bars) received nine patches of three flavours of food, one of which had added quinine. The flavour containing quinine is denoted with a 'q' following the flavour: anise, lemon and mint with quinine (ALMq); anise, lemon with quinine and mint (ALqM); or anise with quinine, lemon and mint (AqLM). There was no effect of either *flavour(s)* or *complexity* on (a) the proportion of larvae choosing the rewarded odour when tested later during the larval stage or (b) the proportion of adult flies choosing the rewarded odour.

Adult Learning Ability

Rearing flies in complex larval foraging environments did not result in greater learning than rearing flies in simple environments (proportion choosing the rewarded odour, respectively: 0.600 ± 0.038 and 0.609 ± 0.037, N = 48; $F_{1,40}$ = 0.02, p = 0.891; Fig. 2b). We did observe significant overall learning, however, as pairing of an odour with sucrose increased later preference for that odour ($F_{1,40}$ = 22.1, p < 0.001), with an overall x proportion of 0.604 ± 0.026 choosing the rewarded odour. We observed a slight preference for MCH, despite preliminary balancing of odour preference, as indicated by a significant effect of *rewarded odour* on the proportion of flies choosing the rewarded odour ($F_{1,40}$ = 10.4, p = 0.003); however, pairing an odour with sucrose increased preference for this odour (MCH preference when MCH+, 0.690 ± 0.030, vs. OCT+, 0.482 ± 0.035). There was no significant effect of the *side of odour presentation* on the proportion of flies choosing the rewarded odour ($F_{1,40}$ = 2.1, p = 0.150). Additionally, the proportion of flies remaining in the centre of the test apparatus (not making a choice) did not differ due to *complexity* ($F_{1,40}$ = 0.2, p = 0.9) or *flavour(s)* ($F_{4,40}$ = 0.8, p = 0.5), with an overall x of 0.180 ± 0.014.

Discussion

We found no evidence for adaptive plasticity of learning ability in fruit flies. Our early-life treatments differed in complexity of foraging environment while controlling for relevant developmental factors such as nutrition and temperature. In our 'complex' environments, we forced the larvae to search, sample and choose from multiple food sources of different bitterness. We thought that these factors represented an ecologically valid manipulation of environmental complexity at the larval stage as larvae attend to such environmental variation, and similar variation has proven effective in previous assays of larval learning

(Dukas 1999; Scherer et al. 2003; Neuser et al. 2005; Gerber & Stocker 2007; Kaun et al. 2007). Furthermore, similar manipulations of food types for varying environmental complexity have been used successfully in a few insect taxa (e.g. Dukas & Real 1993b; Bernays 1998; Gegear & Laverty 2005). Contrary to our prediction, developing in these 'complex' environments did not result in greater learning abilities than 'simple' environments, either later as larvae or as young adults. We did, however, replicate previous studies showing robust learning in both larval and adult fruit flies (reviewed by Gerber et al. 2009), and it is interesting that while larvae attend to food sweetness/bitterness and associated flavours and odours, variation in the complexity of these factors did not noticeably affect the development of later learning ability.

The brains of fruit flies are highly plastic, especially within the mushroom bodies, structures critical for learning and memory, and so they remain a good model for future study of the plasticity of learning. Adult experience with a wide range of social and environmental stimuli can modify neuropil volume and fibre number in the mushroom bodies, but it is unclear how such changes relate to learning ability (Technau 1984; Balling et al. 1987; Heisenberg et al. 1995; Barth & Heisenberg 1997; Fahrbach 2006). Larval mushroom bodies, since they possess functioning neuroblasts (Technau & Heisenberg 1982; Ito & Hotta 1992), may be particularly likely to respond to environmental variation. For instance, Heisenberg et al. (1995) observed that larval development under high density resulted in increased fibre number and larger mushroom bodies at eclosion, although this effect was limited to females. Additionally, both pharmacological and environmental (sporadic heat shock) disruption of larval neurogenesis impairs adult learning and memory (De Belle & Heisenberg 1994; Wang et al. 2007). The question remains whether ecologically relevant environmental factors can cause increased mushroom body neurogenesis or improve learning ability.

Fruit flies may exhibit adaptive plasticity of learning ability for other environmental cues that we did not test. For example, the availability of adequate nutrition may be a more relevant cue of environmental difficulty. In several songbird species, malnutrition is an early-life stressor that results in cognitive deficits of quality and quantity of song learning (Nowicki et al. 2002; Searcy & Nowicki 2009) and spatial memory (Pravosudov et al. 2005; Pravosudov 2009). Although such deficits may be maladaptive in birds (Pravosudov 2009), one can readily imagine a case where an individual experiencing a barely adequate food supply invests more in the cognitive abilities that will better prepare it to find a novel source of food for itself and its offspring. Among fruit flies, it has recently been shown that larval nutritional adversity can affect other foraging related behaviours, increasing the tendency to explore among so-called *sitters* but not *rovers*, flies possessing different variants of the *foraging* gene, for^s and for^R, respectively (Burns et al. 2012). That is, nutritional stress effectively makes the *sitters* more like *rovers*. Interestingly, this gene has also been implicated in a trade-off between short- and long-term memory, with *rovers* possessing better short-term and *sitters* possessing better long-term memory (Kaun et al. 2007). It would be interesting to test whether developmental nutritional stress can adaptively improve the short-term memory of *sitters*. Additionally, cues of predation or competition could trigger an adaptive developmental shift away from learning ability and towards faster development to out-compete others on a dwindling resource or to leave a vulnerable site as soon as possible. Indeed, any environmental cues of expected longevity may be particularly relevant (Eliassen et al. 2007).

Alternatively, fruit flies may not possess adaptive plasticity of learning ability. It could be that the larval food environment is not predictive of the adult's future environment because adults possess greater mobility and can more readily find novel sources of food. Another alternative is that although the larval environment is predictive of the future adult environment, adults utilize their learning abilities in a wide range of contexts, not just foraging. For example, learning plays a role in mate choice (Dukas 2005a,b), and flies may benefit from learning regardless of foraging environment. Finally, the global fitness cost of developing unnecessary brain structures may be small compared to the potential costs of plasticity (DeWitt et al. 1998; Snell-Rood et al. 2010b), such as a developmental error or environmental mismatch. Learning could be so crucial to fitness that instead of being plastic, it is a highly canalized developmental priority regardless of environment (Pravosudov 2009; Roth et al. 2010, 2012).

Despite our failure to find evidence for plasticity of learning ability in fruit flies, our results add to a growing body of literature on the ecology, evolution and development of fruit fly cognitive abilities (Burger et al. 2008; Dukas 2008c; Kolss & Kawecki 2008; Reaume et al. 2010). Fruit flies are an important model system for the neurogenetics, ecology and evolution of learning and memory (Dukas 2008c; Gerber et al. 2009; Busto et al. 2010; Burns et al. 2011), and

our protocols and results are highly relevant for future research. In general, negative results are also important to shed light on the evolution and ecology of cognitive plasticity because we do not expect plasticity to evolve under all conditions (DeWitt et al. 1998; Snell-Rood et al. 2010b). Comparing related species that do and do not exhibit cognitive plasticity in different contexts will further highlight the associated ecological and neurodevelopmental factors.

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