

Substrate choice by ovipositing mothers and consequent hatchling behaviour: the exploration sharing hypothesis



Kevin R. Abbott¹, Reuven Dukas^{*}

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

ARTICLE INFO

Article history:

Received 21 April 2016

Initial acceptance 10 June 2016

Final acceptance 4 July 2016

MS. number: A16-00355

Keywords:

butterfly

Drosophila melanogaster

egg laying

exploration

fruit fly

oviposition

Both maternal and offspring fitness would be higher if the offspring develop in a high-quality habitat than in a low-quality habitat. In animals without direct maternal care, it seems reasonable that either mothers or offspring should invest in finding the best habitat. Recent research, however, suggests extensive maternal and offspring exploration in fruit flies. We considered two hypotheses that could resolve this paradox, first that mothers do not choose oviposition sites that maximize larval success but rather sites that maximize their own fitness or egg survival, and, second, that the winged mothers make decisions over a larger spatial scale than the less mobile larvae are capable of. In a set of experiments, we found that larvae were more likely to explore when they hatched in poor patches lacking protective cavities and live yeast. Egg-laying females, however, also showed strong preferences for ovipositing in cavities with live yeast. These results provided no support for the first hypothesis. We then considered whether females are sensitive to larval travel costs between cavities and live yeast as suggested by the second hypothesis. We found no effect of the distance between the two patches on female oviposition choice, but we did find an effect of the intervening substrate. Females overwhelmingly preferred to lay in the cavity when the intervening substrate was an agar medium, but not when the intervening substrate was bare plastic that discourages larval travel. Therefore, we resolve the paradox of extensive exploration by both mothers and offspring by showing that larvae that hatch in habitats that are desirable to ovipositing females, but not to the larvae, can reasonably assume that there are better food patches within a safe travel distance. That is, there is an adaptive sharing of exploration between mothers and offspring. The exploration sharing hypothesis is pertinent for a large variety of species in which mothers oviposit in sites suboptimal for larval growth.

© 2016 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

There are many systems where females influence the success of their offspring through their choice of where to lay eggs (i.e. oviposition decisions), which can be considered an indirect form of maternal care (Wiklund & Persson, 1983). For example, many insect species that lay their eggs on plants prefer host species that increase offspring success (Gripenberg, Mayhew, Parnell, & Roslin, 2010; Janz, 2002; Refsnider & Janzen, 2010; Schäpers, Nylin, Carlsson, & Janz, 2016; Thompson & Pellmyr, 1991). Insects and amphibians that deposit eggs into water often prefer bodies of water that will not dry up during larval development, that contain suitable food for their offspring and that do not contain competitors, predators or parasites (Refsnider & Janzen, 2010). Similarly, the oviposition decisions of beach-nesting sea turtles can affect the

ability of hatchlings to navigate to the sea (Kamel & Mrosovsky, 2004).

Like more direct examples of parental care, which involve a reallocation of tasks from offspring to parents (e.g. foraging and antipredation tasks), oviposition decisions could be thought of in terms of how exploration or habitat selection tasks are allocated between mothers and offspring (Gamberale-Stille, Söderlind, Janz, & Nylin, 2014; Schäpers et al., 2016; Soler et al., 2012). If mothers reliably oviposit in the best locally available patch for their hatchlings, then the hatchlings should invest little in exploration even if they are in a low-quality patch. However, as in systems with direct parental care, strategies that maximize the parental success are not necessarily identical to the strategy that maximizes the success of a given offspring. Such a parent–offspring conflict could lead to oviposition sites that significantly deviate from the offspring's optimal habitat (Gamberale-Stille et al., 2014; Janz, 2002; Schäpers et al., 2016). This could select for early offspring exploration where hatchlings leave the egg site and search for a suitable feeding site

^{*} Correspondence: R. Dukas, Animal Behaviour Group, Department of Psychology, Neuroscience & Behaviour, McMaster University, Hamilton, ON L8S 4K1, Canada.

E-mail address: dukas@mcmaster.ca (R. Dukas).

¹ E-mail address: abbotkr@mcmaster.ca (K. R. Abbott).

(i.e. the exploration tasks are allocated to the offspring; Wiklund, 1984; Janz, 2002; Gamberale-Stille et al., 2014; Schäpers et al., 2016).

The above discussion suggests that the allocation of exploration effort would vary between species. While this framing is appealing, it does not explain systems where both mothers and offspring explore extensively. Such systems raise two linked questions. Why has natural selection favoured offspring that behave as though they do not trust their mothers' choices? And why has natural selection favoured extensive maternal exploration when the offspring will just leave? A starting point for understanding such a system is to determine why discriminating mothers might not reliably lay their eggs in the best available patch from the hatchling's perspective. We consider two hypotheses for why this might be.

HYPOTHESIS 1: DIVERGING PRIORITIES

While hatchling preferences should be influenced by factors that affect their growth and survival, maternal preferences might be partially or wholly influenced by other priorities. For example, females might choose oviposition sites that directly influence their own fitness (Janz, 2002; Refsnider & Janzen, 2010; Scheirs, Bruyn, & Verhagen, 2000), or pick patches that protect them from predators, provide them with food or other resources, or make it easier for them to place their eggs. Alternatively, females might prioritize oviposition sites that are suitable for eggs rather than hatchlings (Janz, 2002; Refsnider & Janzen, 2010).

HYPOTHESIS 2: SPATIAL SCALE FACTORS

Recently hatched individuals make exploration decisions at a local spatial scale, and therefore, may need to decide whether or not to explore based solely on the conditions surrounding the hatching site. In most species, ovipositing females could consider a much larger spatial scale when choosing where to lay their eggs. More generally, any system where ovipositing females attend to a larger spatial scale than their recently hatched young might lead to different observed preferences that could potentially favour early exploration. Note that such spatial scale factors might interact with Hypothesis 1; females might prefer a specific oviposition site that is consistent with maternal or egg priorities, but only if that site is relatively close to a site that would maximize hatchling success (Refsnider & Janzen, 2010; Soler et al., 2012). For example, butterfly species that lay eggs that will overwinter seem to be inclined to find the larval host plant and then lay the eggs some distance from the host in a location that might be better suited for egg survival (Wiklund, 1984).

One system that may involve extensive maternal and offspring exploration is the fruit fly *Drosophila melanogaster*. Recent published research and personal observations within our laboratory suggest extensive maternal and larval exploration. In particular, Yang, Belawat, Hafen, Jan, and Jan (2008) suggested that females extensively sample a potential egg-laying substrate before laying each egg, and a number of studies have shown that females are discriminating in where they lay their eggs (Durisko, Anderson, & Dukas, 2014; Golden & Dukas, 2014; Mery & Kawecki, 2002; Miller et al., 2011; Rodrigues et al., 2015; Sarin & Dukas, 2009; Schwartz, Zhong, Bellemer, & Tracey, 2012; Yang et al., 2008). Similarly, several studies have shown that larvae are quite mobile and are inclined to seek out better substrates (Durisko & Dukas, 2013; Rodrigues et al., 2015; Schwartz et al., 2012; Schwarz, Durisko, & Dukas, 2014). Finally, our preliminary observations indicated that newly hatched larvae engage in extensive exploration even when they are on high-quality media.

Our strategy was to first determine whether recently hatched *D. melanogaster* larvae do explore when they hatch in patches of different qualities. We then tested the hypotheses proposed above for why the newly hatched larvae might act as though they do not trust their mothers. We started by testing a prediction derived from Hypothesis 1: egg-laying females and recently hatched larvae will show different patch preferences when presented with choices at a similar spatial scale.

EXPERIMENT 1: EXPLORATION IN RECENTLY HATCHED LARVAE

Methods

Our exploration arenas were 35 mm petri dishes, each filled with 5 ml of medium containing agar (22 g/litre), cornmeal (83 g/litre) and orange juice concentrate (204 g/litre) and containing a central patch 5 mm in diameter. We used a 2 × 2 factorial design to vary two patch features attractive to fruit fly larvae, live yeast and a cavity (Fig. 1a). Hence the four patch treatments were yeasted cavity, yeast, cavity and plain. The yeasted cavity patches involved a 2.5 µl drop of live yeast suspension (0.6% weight/volume active dry baker's yeast in warm water plus one drop of red food colouring per ml solution) in a 5 × 5 mm depression (created by removing an inverted pyramid-shaped piece of the medium with a spatula). The yeast patches were created with the yeast solution without the cavity. The cavity patches consisted of a cavity and a 2.5 µl drop of red food colouring solution (one drop of food colouring per ml of warm water). The plain patches contained only a 2.5 µl drop of red food colouring solution.

Subjects for this and all subsequent experiments were from a Canton-S population that has been maintained under standard conditions in our laboratory for 7 years (Sarin & Dukas, 2009). Our flies are kept at low density in large Plexiglas cages at an ideal temperature (25 °C) and high humidity (60%), and provided regularly with fresh food optimized for larval growth and adult survival and reproduction. We transferred a single recently laid egg to the centre of each patch of the exploration arenas and kept them in chambers at 25 °C and high humidity (>90% RH). The following day, we monitored the arenas over the period of peak larval hatching. While we wished to observe the movement decisions of hatchlings based only on their hatching environment, our preliminary experiments suggested that the hatchlings responded to our presence. We thus designed a protocol that minimized observer interference. We scanned for hatched eggs in full darkness using a 10x magnifying glass and a flashlight equipped with a filter transmitting red light above 600 nm (Rosco Roscolux no. 27, medium red), which is beyond the visible spectrum of the larvae (Keene & Sprecher, 2012). Following hatching, we moved the dishes and covered them with tinfoil muffin cups, where the larvae remained undisturbed in full darkness for 30 min. We then placed the dishes on ice to arrest larval movement and later transferred them into a –20 °C freezer. After a minimum of 24 h in the freezer, we thawed the exploration arenas and determined whether larvae were within the central patch (residents) or away from the patch (explorers). The central patch was not always visible in the plain arenas, so for consistency, in all conditions, we defined patch residency in terms of distance of the larvae from the egg casing such that larvae that were <5 mm from the egg casings were classified as residents and larvae that were ≥5 mm were classified as explorers. We preserved the exploration arenas of 111 hatchlings, but our final sample size included only 96 arenas because we failed to locate the larvae in 15 arenas (see Results). We analysed

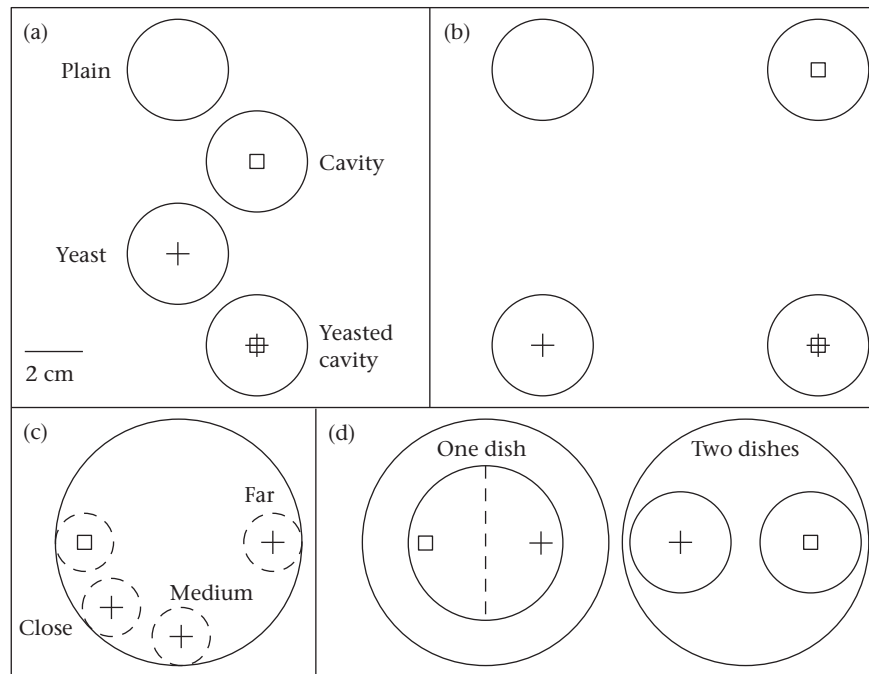


Figure 1. Schematic of the experimental designs, which are all drawn to scale and use the same symbols as noted in (a). (a) The four dish types used in experiment 1. Individual eggs were placed on the central patch of a given dish type and the displacements of hatchlings (30 min old) were noted. (b) Relative placement of the four dishes in experiment 2. The dish types were the same as in experiment 1. Recently mated females were placed in a cage (not shown) with all four dish types (order was randomized) and the location of eggs was noted. (c) Relative placement of the cavity and the live yeast patches in experiment 3. Recently mated females were placed in a dish with a cavity and one of three live yeast patches (close, medium or far, relative to the cavity). Numbers of eggs within the regions (dashed circles) of the cavity and the live yeast patch and elsewhere on the dish were noted. (d) Placement of dishes and patches in the two treatments of experiment 4. Recently mated females were placed in a dish with the cavity and the live yeast patches in either one or two dishes. Numbers of eggs within both patch regions (one-dish condition; regions indicated by dashed line) or within both dishes (two-dish condition) were noted.

the data with a generalized linear model (GLM) with binomial distribution and logit link function.

To quantify the effectiveness of our method of arresting larval movement, we made dishes identical to the plain exploration arenas and added one first-instar larva to the centre of each patch. We immediately placed half the dishes on ice while keeping the other half at the room temperature of 25 °C. After 10 min, we measured (to the nearest mm) how far each larva was from the edge of the central patch. While 90% of the larvae in the ice group were within the central patch, 100% of the larvae in the control group were away from the patch (mean distance from the patch of 0.1 mm and 8.2 mm for the ice and control groups, respectively; Mann–Whitney U test: $U = 0.0$, $N_1 = N_2 = 10$, $P < 0.0001$). Further observations indicated that the effect of ice was almost instantaneous and that larvae likely move less than a body length after being placed on ice.

Results

There was a strong effect of condition on the proportion of larvae that explored (likelihood ratio: $\chi^2_3 = 55.1$, $N = 96$, $P < 0.001$; Fig. 2a). There was a main effect of both cavity and yeast, such that either reduced exploration, but no statistically significant interaction (GLM with $N = 96$; cavity: Wald: $\chi^2_1 = 6.7$, $P = 0.01$; yeast: Wald: $\chi^2_1 = 26.2$, $P < 0.001$; cavity*yeast: Wald: $\chi^2_1 = 0.003$, $P = 0.95$). We had to omit from the analysis 15 arenas in which we failed to locate the larvae, and all but one of these arenas had cavities (cavity or yeasted cavity treatment). Given the ease at which we found larvae out of the cavities, it is likely that all of these undetected larvae were in the cavity and this potentially biased the results. Therefore, as a conservative measure, we

reanalysed the data while adding larvae from these 14 cavity-containing arenas into the resident category. This analysis was similar to the one above (GLM with $N = 111$; Omnibus test: likelihood ratio: $\chi^2_3 = 58.5$, $P < 0.001$; cavity: Wald: $\chi^2_1 = 10.8$, $P = 0.001$; live yeast: Wald: $\chi^2_1 = 23.5$, $P < 0.001$; cavity*live yeast: Wald: $\chi^2_1 = 0.2$, $P = 0.67$).

EXPERIMENT 2: PREFERENCES OF OVIPOSITING FEMALES AMONG FOUR PATCHES

Rationale

Experiment 1 confirmed that early exploration exists in fruit fly larvae and that it is contingent such that it is more likely to occur when larvae hatch in low-quality patches. An implication of this result is that larvae do not ‘assume’ that their mothers’ egg-laying choice represents the best locally available patch. It is possible that this reflects the fact that ovipositing females are not very good at assessing patch quality relative to hatchlings. However, recent results suggest that females are discriminating in their choice of oviposition site (see Introduction). Thus, another possible explanation is that, while females are discriminating in their choice of oviposition sites, they are making their choices based on different factors than the larvae (Hypothesis 1). Both of these hypotheses (poorer maternal than larval patch assessment and divergent criteria of mothers and offspring) predict that the egg-laying choices of females will be distinct from the hatchling preferences as inferred from experiment 1. We tested this prediction by allowing females to lay eggs on the same food substrates as in experiment 1.

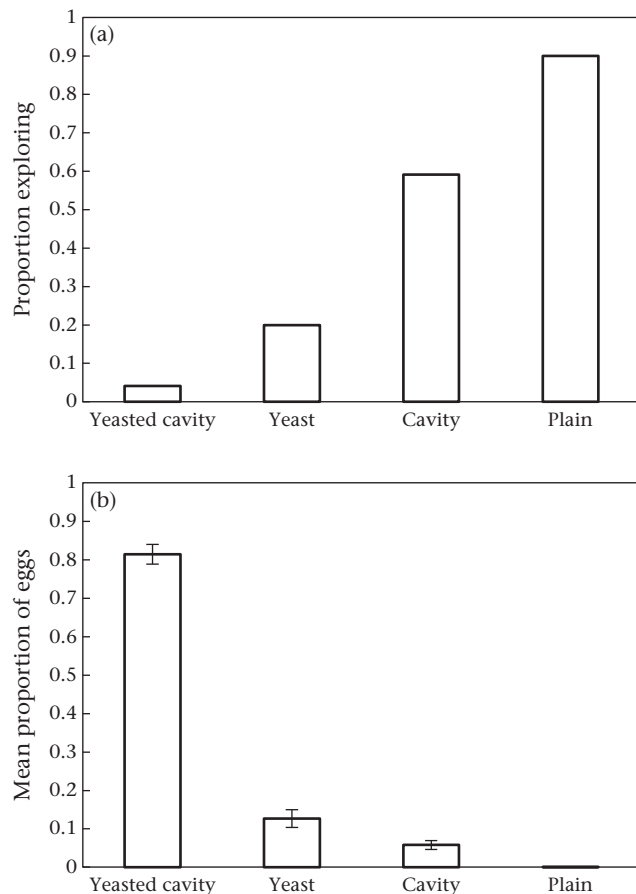


Figure 2. Larval and maternal decisions. (a) Proportion of recently hatched larvae in each of the four treatment combinations that were found ≥ 5 mm from their egg casing after 30 min of potential exploration time. The sample sizes for each treatment combination was 24 (yeasted cavity), 20 (yeast), 22 (cavity) and 30 (plain). (b) Mean proportion \pm SEM of eggs laid on each treatment combination by females that had access to all four treatment combinations. $N = 117$.

Methods

We used the same food, dishes and manipulations (cavities and yeast suspensions) as in experiment 1 and the basic set-up for studying oviposition decision as in [Sarin and Dukas \(2009\)](#). We placed all four types of dishes in each cage measured $23 \times 13 \times 18$ cm ($l \times w \times h$) and arranged them in a 9.5×9.5 cm square with positions randomized ([Fig. 1b](#)). We added a recently mated 4-day-old female to each cage and left females in the cages overnight (as defined by the light cycle they were kept on: 2200–1000 hours). We then counted the number of eggs on these dishes. We ran 120 cages but excluded one cage from the analyses because it had two flies in it and two cages where no eggs were laid. We analysed the data with a generalized linear model (GLM) with gamma distribution and log link function and female as a repeated measure within each cage.

Results

There was a main effect of both cavity and yeast, such that either factor increased the number of eggs laid on a dish (cavity: Wald: $\chi_1^2 = 190$, $P < 0.001$; live yeast: Wald: $\chi_1^2 = 237$, $P < 0.001$; [Fig. 2b](#)). There was also a statistically significant interaction because the live yeast and cavity had a synergistic effect on the number of eggs laid on the yeasted cavity dish (Wald: $\chi_1^2 = 31.5$, $P < 0.001$).

EXPERIMENT 3: PATCH PREFERENCES OF OVIPOSITING FEMALES AS A FUNCTION OF DISTANCE

Rationale

Experiments 1 and 2 indicated rather similar patch preferences by ovipositing females and hatchlings. For example, both mothers and offspring seemed to show a strong dislike for the plain treatment relative to the other dish types; in experiment 1, 90% of the larvae had left the plain natal patch, and, in experiment 2, only 1% of the females laid eggs in the plain dish. Thus we found no evidence in support of Hypothesis 1, which states that hatchlings explore because their mothers choose oviposition sites that are unsatisfactory to the hatchlings.

Given the lack of support for Hypothesis 1, we moved on to considering the spatial scale of the decisions made by hatchlings and ovipositing females (Hypothesis 2). Specifically, we looked for evidence that females change their oviposition preferences as a function of the distance between a cavity and a patch of live yeast, which would suggest that females are making their decisions based on a large spatial scale. Our prediction was that females would prefer the cavity when it was close to the live yeast but would show a greater preference for the live yeast patch when it was farther from the cavity. This prediction was based on two facts: it is easier to lay eggs in the broken surface of a cavity, and live yeast is essential for larval development.

Methods

We used the same medium as in previous experiments but poured it into 88 mm diameter petri dishes. We created two patches equivalent to the cavity and yeast conditions in previous experiments. The centres of these patches were 10 mm from the edge of the dish and 28, 48 or 68 mm from each other (close, intermediate and far conditions, respectively; [Fig. 1c](#)). We placed each 88 mm dish inside a larger 100 mm dish inside a high humidity chamber as in experiment 1. We added a single recently mated female to each larger dish through a sealable hole in the lid and allowed them to lay eggs overnight as in experiment 2. We then counted the number of eggs in a 20 mm diameter circle surrounding each of the patches as well as in the rest of the dish. We ran 40 females in each condition. Females laid similar number of eggs in the different conditions (one-way ANOVA: $F_{2,117} = 0.13$, $P = 0.88$). The dependent variable for our test of the effect of distance on female oviposition preference was the proportion of eggs laid in the cavity region relative to the total number of eggs laid in the two focal regions. We excluded the seven cages where no eggs were laid (one cage in the intermediate condition and three cages in each of the near and far conditions). We used a generalized linear model with gamma distribution and identity link.

Results

The distance between the cavity and yeast had no effect on female oviposition preference (Wald: $\chi_2^2 = 0.80$, $P = 0.67$; [Fig. 3](#)).

EXPERIMENT 4: PATCH PREFERENCES OF OVIPOSITING FEMALES AS A FUNCTION OF INTERVENING SUBSTRATE

Rationale

Experiment 3 provided no evidence that females attend to the distance between relevant resources when choosing whether to oviposit in a cavity or a patch of yeast. As a follow-up experiment, we wished to examine whether females do consider the distance

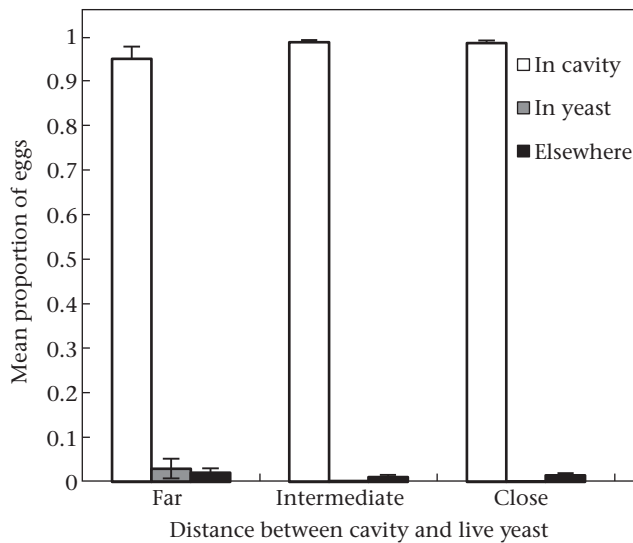


Figure 3. Oviposition decisions as a function of distance. Mean proportion \pm SEM of eggs laid in the cavity patch, in the yeast patch, or elsewhere on the dish when the distance between the patches was 28 mm (close; $N = 37$), 48 mm (intermediate; $N = 39$) or 68 mm (far; $N = 37$).

between resources when they are separated by a medium hostile to larval travel rather than the hospitable moist agar used in experiment 3. Hence, in experiment 4 the resources (cavities and yeast) were always the same distance apart but were either in the same dish separated by moist agar or in separate dishes separated by plain plastic. We predicted that the females would lay a higher proportion of their eggs at the yeast patch when it was separated from the cavity by plain plastic than by moist agar.

Methods

The protocol was effectively the same as experiment 3 except that, instead of filling the 88 mm diameter dish with food medium, we placed either one 53 mm or two 35 mm diameter petri dishes of food into the 88 mm dish (Fig. 1d). We selected the two dish sizes so that the total surface area available for egg laying was approximately equivalent in the two conditions (2206.18 mm² in the one-dish condition and 1924.23 mm² in the two-dish condition). In the one-dish condition, the cavity and live yeast were on opposite edges of the dish and 45 mm from each other. The dishes in the two-dish condition were identical to the cavity and live yeast dishes in experiments 1 and 2. These two dishes were on opposite sides of the 88 mm dish such that the two resources were 45 mm apart and there was a 10 mm distance between the two dishes. We ran 150 dishes evenly split between the two conditions. We counted all eggs laid as being either in the cavity region or in the live yeast region. In the one-dish condition, this involved dividing the dish in two halves; in the two-dish condition, this involved counting all the eggs in either dish as belonging to the appropriate resource type. The data were bimodal both in terms of the total number of eggs laid (there was a second peak at 0) and in terms of where the eggs were laid (in the two-dish conditions there was a relatively high frequency of females that showed an absolute preference for either the live yeast dish or the cavity dish). Therefore, we used nonparametric analyses. Females laid similar numbers of eggs in the two conditions (40.6 ± 2.8 and 40.2 ± 3.2 in the one- and the two-dish conditions, respectively; Mann–Whitney U test: $U = 2752.0$, $N_1 = N_2 = 75$, $P = 0.82$). The dependent variable for our test of the effect of intervening substrate on female oviposition preference was the proportion of eggs laid in the cavity region and

we omitted cases where the female laid no eggs (12 cases in the one-dish condition, 13 cases in the two-dish condition).

Results

Females laid a higher proportion of eggs on the live yeast under the two-dish condition where the cavity and yeast were separated by plain plastic than in the one-dish condition where the resources were separated by moist agar (Mann–Whitney U test: $U = 3241.5$, $N_1 = 63$, $N_2 = 62$, $P < 0.001$; Fig. 4).

DISCUSSION

Explaining Failures of the Preference Performance Hypothesis

A lot of research on insect oviposition decisions has focused on the preference–performance hypothesis, and more specifically on the fact that the hypothesis fails in many systems where females do not particularly prefer to lay eggs in sites that maximize offspring performance (Gripenberg et al., 2010; Janz, 2002; Refsnider & Janzen, 2010; Thompson & Pellmyr, 1991). This hypothesis assumes that the mother's oviposition decisions function to maximize larval success, so one class of solution to the frequent failure of this hypothesis is to consider other fitness components that the mother might be trying to maximize (Janz, 2002; Refsnider & Janzen, 2010). She may be trying to maximize her own fitness at the expense of offspring success (e.g. lay more eggs even if each offspring has a lower chance of survival), or she may be choosing a site that maximizes offspring success over all life stages (e.g. egg, various larval instars, pupae, adult). These alternatives to the preference–performance hypothesis could be called the preference–fitness hypothesis as it predicts that females would prefer to lay eggs in sites that maximize multiple fitness components. The preference–fitness hypothesis is reasonable and does explain many systems but, like the preference–performance hypothesis, it ignores the fact that offspring can be active participants in their own success. Thus another class explanation for situations where mothers do not choose in a way that maximizes larval performance focuses on the idea that the exploration tasks may be allocated to the offspring (Gamberale-Stille et al., 2014; Schäpers et al., 2016; Soler et al., 2012; Wiklund, 1984). This could be called the

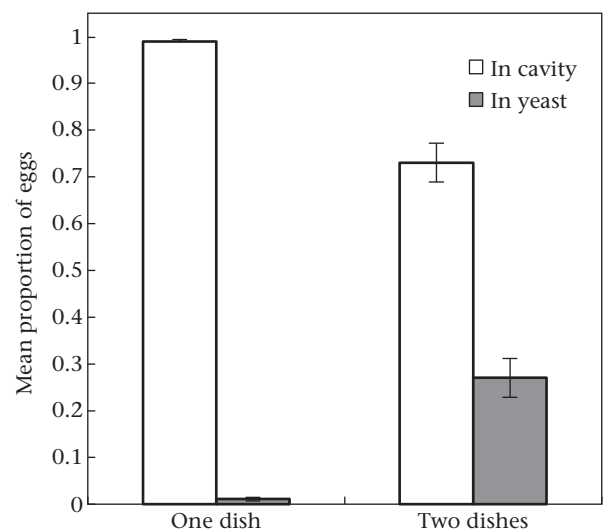


Figure 4. Oviposition decisions when resources were in one dish or two dishes. Mean proportion \pm SEM of eggs laid in the cavity, in the yeast region (one-dish condition; $N = 63$), or in the dish (two-dish condition; $N = 62$).

exploration allocation hypothesis. The selective forces that allocate the exploration task to mothers or offspring can be thought of in terms of mother–offspring conflict. Alternatively, the allocation of exploration could be an outcome of the fact that one generation or the other might be fundamentally more capable and efficient in terms of exploration. The fact that mothers are generally more mobile than their offspring suggests that mothers will often be more capable and efficient explorers. However, it could well be that the offspring, and not the mothers, have the sensory capabilities to assess the relevant features of their environment. Furthermore, offspring may be better at making the habitat selection decision for the simple fact that they are the ones present when the decision has to be made. In overwintering eggs, for example, larvae in the spring are likely better suited to make microhabitat decisions than are their mothers given that much could change over a winter (Wiklund, 1984). Similarly, in fruit flies, conditions in a decaying fruit patch can change rapidly and unpredictably as a function of the composition and abundance of both the microbial community and the fruit fly larvae that feed on some of these microbes (Rohlf, Obmann, & Petersen, 2005; Stamps, Yang, Morales, & Boundy-Mills, 2012; Venu, Durisko, Xu, & Dukas, 2014). Thus an egg that was laid in a high-quality patch may hatch in a low-quality patch. Similarly, nearby patches that were rejected by the female as low-quality may have increased in quality in the intervening time.

We found evidence of early exploration in *D. melanogaster* larvae, but we also found evidence that the mothers are very discriminating and seem to choose the sites that are associated with low rates of larval exploration (Fig. 2). Early offspring exploration can explain failures of the preference–performance hypothesis, but it is odd in systems where the mothers seem to behave in accordance with the preference–performance hypothesis. However, we also found evidence that ovipositing females respond to features at a larger spatial scale than would be possible for hatchlings (Hypothesis 2). When a patch of yeast and a cavity were separated, females' decisions depended on the substrate that the larvae would have to travel to get from the cavity to the yeast. When the intervening substrate was conducive to larval travel (moist agar), females overwhelmingly chose to lay their eggs in the cavity, but when the intervening substrate was less conducive to larval travel, more females chose to lay eggs in the yeast patch (Fig. 4). This suggests a third class of explanation for the frequent failure of the preference–performance hypothesis, which essentially combines the arguments of the preference–fitness and exploration allocation hypotheses. This exploration sharing hypothesis is like the preference–fitness hypothesis in suggesting that females choose a specific oviposition site based on factors other than just larval success (particularly female direct fitness and egg success). However, the exploration sharing hypothesis also suggests that the cost of these maternal decisions to larval success is mitigated by larval exploration. This sharing of exploration tasks might be most obvious when spatial scale is considered. The maternal share of exploration might involve finding an area of larger scale that contains distinct sites that maximize all of the fitness components. However, within that larger-scale area, she might oviposit in the site that maximizes maternal and egg fitness components. The larval share of the exploration would then involve finding the site within this larger-scale area that maximizes larval, pupal and eclosing adult fitness components. As in our results, the maternal share of exploration may involve considering the larval travel costs between the two sites in this larger-scale area. The simplest mechanism that can allow female fruit flies to effectively consider larval travel costs in their oviposition decisions is an integration of both the value of the prospective egg-laying spot and the value of its surrounding substrate. Such integration will reduce the overall value of a good site surrounded by a substrate that

hinders larval travel. This hypothesis may be critically tested using established neurogenetic protocols (Kacsoh, Lynch, Mortimer, & Schlenke, 2013; Yang et al., 2008).

Note that the possibility that ovipositing females consider the larval travel costs could affect the conclusions that can be drawn from previous tests of the preference–performance hypothesis. In many such experiments (see supplementary material in Gripenberg et al., 2010), researchers identify plants on which larvae do or do not thrive. They then give the mothers a simultaneous choice between these plants. If mothers perceive the distance between these plants as being traversable for their offspring, then they might be less motivated to lay eggs on the plant that maximizes larval performance. This concern may also apply to field observations of eggs on different plant types; depending on the distribution of the plant types and on larval mobility, laying eggs on a given plant type may have little effect on where the larvae develop. In both experimental set-ups, a lack of preference for performance-enhancing plants is not necessarily evidence that mothers do not consider features of the environment that affect larval performance; the mothers may lay their eggs near, but not on, the performance-enhancing plants. This is not to say that simultaneous choice tests are not useful. They make sense for systems with immobile offspring and they make sense for studies of how maternal and/or larval behaviours contribute to the end goal of getting the larvae to a good site for development in naturalistic patchy habitats (Gamberale-Stille et al., 2014; Schäpers et al., 2016; Soler et al., 2012).

Oviposition Decisions in Fruit Flies

Previous research has suggested that female fruit flies consider larval travel costs in their oviposition decisions (Miller et al., 2011; Schwartz et al., 2012; see also the discussion of hierarchical decision making in; Refsnider & Janzen, 2010), but this study is unique in demonstrating that the intervening substrate matters, not just the distance between patches. This makes sense given the natural ecology of fruit flies (*D. melanogaster*) where eggs are laid on rotting fruits on the ground (Spieth, 1974; Sturtevant, 1921). These rotting fruits act like patches of varying sizes and qualities, and while it may be that larvae can always move easily within such a patch, movement between patches likely depends on the nature of the ground cover. Thus a female that is deciding between a patch that is better for herself or her eggs and a patch that is better for her larvae will need to assess the feasibility and costs of larval movement between patches.

The Exploration Allocation Hypothesis

While this study suggested that exploration is shared between the generations rather than primarily allocated to one generation or the other, we still think the exploration allocation hypothesis is worth further consideration. Of particular interest is the idea that there might be an intergenerational–exploitation trade-off such that if mothers explore more, larvae can spend less time exploring and more time exploiting their habitat and vice versa (see Cohen, McClure, & Yu, 2007 for the more traditional individual-level framing of the exploration–exploitation trade-off). There is some limited evidence that larvae show less exploratory tendencies or abilities in butterfly species where mothers are more discriminating in their oviposition decisions, but this conclusion is based on a small number of species (Schäpers et al., 2016). It is also possible that such an intergenerational exploration–exploitation trade-off could manifest at an individual level such that factors (e.g. genotype, maternal body condition, environmental conditions) that discourage maternal

exploration encourage offspring exploration and vice versa. Presumably for adaptations like this to evolve, there would have to be some cue available to the offspring that suggests the extent of maternal exploration. This cue could be the larval genotype, but given the dilution associated with paternal genotype, this mechanism would probably require maternally imprinted genes. We know of no evidence of an individual-level intergenerational exploration–exploitation trade-off in oviposition decisions, but there is some suggestive, if contradictory, evidence in Gamberale-Stille et al. (2014). In contrast to the idea of an individual-level intergenerational exploration–exploitation trade-off in oviposition decisions, they found that the larval offspring of mothers who were more willing to oviposit on low-quality plants were also more willing to accept the same low-quality plants. Conversely, larvae that were more likely to leave a poor-quality site and find a higher-quality site developed into adults that were less discriminating in their oviposition decisions. The authors suggest that more mobile larvae develop into less discriminating adults, which is consistent with an intergenerational exploration–exploitation trade-off at the individual level. Given the inconsistent results, however, more work would have to be done before any conclusion could be drawn.

Acknowledgments

We thank Milica Stojanovic, Bradley Andrade and Joseph Mentlik for help collecting data for this project, two anonymous referees for thoughtful comments and the Natural Sciences and Engineering Research Council of Canada, Canada Foundation for Innovation and Ontario Ministry of Research and Innovation for funding. K.A. was supported by the E.B. Eastburn Postdoctoral Fellowship from the Hamilton Community Foundation.

References

- Cohen, J. D., McClure, S. M., & Yu, A. J. (2007). Should I stay or should I go? How the human brain manages the trade-off between exploitation and exploration. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 362, 933–942.
- Durisko, Z., Anderson, B., & Dukas, R. (2014). Adult fruit fly attraction to larvae biases experience and mediates social learning. *Journal of Experimental Biology*, 217, 1193–1197.
- Durisko, Z., & Dukas, R. (2013). Attraction to and learning from social cues in fruitfly larvae. *Proceedings of the Royal Society B: Biological Sciences*, 280, 1–7.
- Gamberale-Stille, G., Söderlind, L., Janz, N., & Nylin, S. (2014). Host plant choice in the comma butterfly: Larval choosiness may ameliorate effects of indiscriminate oviposition. *Insect Science*, 21, 499–506.
- Golden, S., & Dukas, R. (2014). The value of patch-choice copying in fruit flies. *PLoS One*, 9, e112381.
- Gripenberg, S., Mayhew, P. J., Parnell, M., & Roslin, T. (2010). A meta-analysis of preference–performance relationships in phytophagous insects. *Ecology Letters*, 13, 383–393.
- Janz, N. (2002). Evolutionary ecology of oviposition strategies. In M. Hilker, & T. Meiners (Eds.), *Chemoeology of insect eggs and egg deposition* (pp. 349–376). Berlin, Germany: Blackwell.
- Kacsoh, B. Z., Lynch, Z. R., Mortimer, N. T., & Schlenke, T. A. (2013). Fruit flies medicate offspring after seeing parasites. *Science*, 339, 947–950.
- Kamel, S. J., & Mrosovsky, N. (2004). Nest site selection in leatherbacks, *Dermochelys coriacea*: Individual patterns and their consequences. *Animal Behaviour*, 68, 357–366.
- Keene, A. C., & Sprecher, S. G. (2012). Seeing the light: Photobehavior in fruit fly larvae. *Trends in Neurosciences*, 35, 104–110.
- Mery, F., & Kawecki, T. J. (2002). Experimental evolution of learning ability in fruit flies. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 14274–14279.
- Miller, P. M., Saltz, J. B., Cochrane, V. A., Marcinkowski, C. M., Mobin, R., & Turner, T. L. (2011). Natural variation in decision-making behavior in *Drosophila melanogaster*. *PLoS One*, 6, e16436.
- Refsnider, J. M., & Janzen, F. J. (2010). Putting eggs in one basket: Ecological and evolutionary hypotheses for variation in oviposition-site choice. *Annual Review of Ecology, Evolution, and Systematics*, 41, 39–57.
- Rodrigues, M. A., Martins, N. E., Balancé, L. F., Broom, L. N., Dias, A. J. S., Fernandes, A. S. D., et al. (2015). *Drosophila melanogaster* larvae make nutritional choices that minimize developmental time. *Journal of Insect Physiology*, 81, 69–80.
- Rohlf, M., Obmann, B., & Petersen, R. (2005). Competition with filamentous fungi and its implication for a gregarious lifestyle in insects living on ephemeral resources. *Ecological Entomology*, 30, 556–563.
- Sarin, S., & Dukas, R. (2009). Social learning about egg-laying substrates in fruitflies. *Proceedings of the Royal Society B: Biological Sciences*, 276, 4323–4328.
- Schäpers, A., Nylin, S., Carlsson, M. A., & Janz, N. (2016). Specialist and generalist oviposition strategies in butterflies: Maternal care or precocious young? *Oecologia*, 180, 335–343.
- Scheirs, J., Bruyn, L. D., & Verhagen, R. (2000). Optimization of adult performance determines host choice in a grass miner. *Proceedings of the Royal Society B: Biological Sciences*, 267, 2065–2069.
- Schwartz, N. U., Zhong, L., Bellemer, A., & Tracey, W. D. (2012). Egg laying decisions in *Drosophila* are consistent with foraging costs of larval progeny. *PLoS One*, 7, e37910.
- Schwarz, S., Durisko, Z., & Dukas, R. (2014). Food selection in larval fruit flies: Dynamics and effects on larval development. *Naturwissenschaften*, 101, 61–68.
- Soler, R., Pineda, A., Li, Y., Ponzio, C., van Loon, J. J. A., Weldegergis, B. T., et al. (2012). Neonates know better than their mothers when selecting a host plant. *Oikos*, 121, 1923–1934.
- Spieth, H. T. (1974). Courtship behavior in *Drosophila*. *Annual Review of Entomology*, 19, 383–406.
- Stamps, J. A., Yang, L. H., Morales, V. M., & Boundy-Mills, K. L. (2012). *Drosophila* regulate yeast density and increase yeast community similarity in a natural substrate. *PLoS One*, 7, e42238.
- Sturtevant, A. H. (1921). *The North American species of Drosophila*. Washington, D.C: Carnegie Institution of Washington.
- Thompson, J. N., & Pellmyr, O. (1991). Evolution of oviposition behavior and host preference in *Lepidoptera*. *Annual Review of Entomology*, 36, 65–89.
- Venu, I., Durisko, Z., Xu, J. P., & Dukas, R. (2014). Social attraction mediated by fruit flies' microbiome. *Journal of Experimental Biology*, 217, 1346–1352.
- Wiklund, C. (1984). Egg-laying patterns in butterflies in relation to their phenology and the visual apparency and abundance of their host plants. *Oecologia*, 63, 23–29.
- Wiklund, C., & Persson, A. (1983). Fecundity, and the relation of egg weight variation to offspring fitness in the speckled wood butterfly *Pararge aegeria*, or why don't butterfly females lay more eggs? *Oikos*, 40, 53–63.
- Yang, C., Belawat, P., Hafen, E., Jan, L. Y., & Jan, Y. (2008). *Drosophila* egg-laying site selection as a system to study simple decision-making processes. *Science*, 319, 1679–1683.