Gene-Environment Interplay on Oviposition Site Selection in *Drosophila melanogaster*

by

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Abstract

The underlying mechanisms of decision-making behaviour are poorly understood. Oviposition site selection (OSS) in the *Drosophila melanogaster* fruit fly provides a useful system for studying simple decision-making. OSS is influenced by both genetic and environmental variation. Variation of the *foraging* gene (*for*) is known to affect OSS. Given a choice of high- and low-nutrient oviposition sites, groups of rovers (*for*^R^) lay significantly more of their eggs on low-nutrient sites than sitters (*for*^s^) and sitter mutants (*for*^s2^). I examined the roles of choice, female density, and *for* expression in gustatory neural circuitry in rover/sitter differences in OSS. I found a role of choice and female density in rover/sitter differences, and a role of *for* in response to glycerol, an indicator of yeast. However, driving *for*-mRNA expression in gustatory neurons using the *GAL4/UAS* system did not alter OSS. These findings suggest a central role of *for* in decision-making during OSS.
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Introduction

Disputes on the mechanisms underlying behaviour have persisted since long before the discovery of the gene or Charles Darwin’s theory of evolution. As part of the debate more contemporarily known as nature versus nurture, evidence of this discussion can be seen as far back as the 17th century, with ideas of animal behaviour being dictated by either instinct or sensation (Herrnstein, 1998; Richards, 1979). While the nature versus nurture debate is commonly seen today to represent genes versus environment, this bares a striking similarity to instinct (nature) versus sensation (nurture). Early proponents of instinct would argue that animal behaviours are innate, lacking any need for cognition, whereas sensationalists would argue that animal behaviours are learned and conditioned by the environment and past experiences, invoking a necessity for such cognitive capabilities as memory (Richards, 1979).

Fortunately, it is now commonly accepted that behaviours, as with physical traits, are influenced by a combination of genes and the environment. This is referred to as gene-environment interaction or interplay. The field of behaviour genetics was created in an attempt to elucidate the relative contributions of genes and environment to behaviours. Francis Galton is considered by many to be the first behaviour geneticist. Galton was responsible for the more contemporary formalization of the nature-nurture dichotomy, and published the first behaviour genetics study, using familial relations to examine the hereditary quality of intelligence, or “genius,” in humans (Fancher, 2009; Galton, 1865; Galton, 1874; Greenspan, 2004; 2008). This marked the beginning of the quantitative genetic approach to behaviour genetics.
Today, the quantitative genetic approach allows for the identification of the approximate number and genomic location of genetic loci contributing to variation in a particular phenotype of interest. A prerequisite for this approach is pre-existing natural heritable variation in a trait, though artificial selection has uncovered heritable variation in most behaviours attempted in this way, including orientation to visual stimuli, learning, movement relative to wind, and courtship song in *Drosophila*, and aggression and wheel-running activity in mice (Carter *et al*., 2000; Gotz, 1970; Greenspan, 2003; 2004; Marden *et al*., 1997; Medioni *et al*., 1978; Ritchie & Kyriacou, 1996; van Oortmerssen & Bakker, 1981; Weber, 1996). Jerry Hirsch was one of the first researchers to demonstrate the use of artificial selection to compare behavioural extremes in his study of geotaxis (movement relative to gravity) in the fruit fly, *Drosophila melanogaster* (Hirsch & Tryon, 1956) and later used selected lines to demonstrate contributions of all major *D. melanogaster* chromosomes to variation in geotaxis (Hirsch & Erlenmeyer-Kimling, 1962; Hirsch & Ksander, 1969; Ricker & Hirsch, 1988). While these studies take into account the effects of multiple loci on traits influenced by multiple genes (polygenic traits) and identify genetic elements important in an evolutionary context, they have traditionally not had the resolution of single genes. This was addressed with the single-gene approach.

Pioneered by Seymour Benzer, the single-gene approach to behaviour genetics uses laboratory-induced mutations to identify genes involved in behaviour (Benzer, 1967; 1973; Greenspan, 2004). While these studies were traditionally not concerned with natural variation or the effects of multiple loci, they benefited from an increased, gene-level resolution. One of the first and most influential studies to use this approach detailed
the role of the *period* gene (*per*) in circadian rhythms of eclosion and locomotor
behaviour in *D. melanogaster* (Konopka & Benzer, 1971). The single-gene approach
made possible the characterization of several other circadian rhythm genes (*timeless,*
*doublesex,* Taylor *et al.*, 1994), and learning and memory genes (*dunce,* Dudai *et al.,*
1976; *amnesiac,* Quinn *et al.*, 1979) in *D. melanogaster* (reviewed in Greenspan, 2004;
Sokolowski, 2001).

Both approaches have become central to the study of behaviour genetics. It is now
commonly accepted that normal individual differences in behavioural traits are generally
polygenic, necessitating the genome-scale focus of the quantitative approach. Some
exceptions, in the form of single genes of large effect, have been documented, including
the *foraging* gene of *D. melanogaster* and the *npr-1* gene of *Caenorhabditis elegans* (de
Bono & Bargmann, 1998; Sokolowski, 1980). Single-gene manipulations can be used to
verify findings from quantitative genetic study, and expand on physiological functions of
the identified genes. It is also recognized that many genes underlying variation in
behaviour influence multiple traits (pleiotropy), further highlighting the complexities of
the genetic structure underlying behaviour. Together, these approaches allow for the
investigation of complex gene networks underlying behaviors (Greenspan, 2004).

The ability to perform artificial selection on most behavioural traits, and generate
inbred and mutants strains propelled behaviour genetics research in animal models
forward, with some of the most prominent model organisms being mice, *D. melanogaster*
fruit flies, and *C. elegans* nematodes (Avery, 2010; Bucan & Abel, 2002; Sokolowski,
2001; Wolinsky & Way, 1990). Despite having relatively simple nervous systems
(100,000 neurons in the *Drosophila* brain compared to an estimated 85 billion neurons in the human brain), many of these model organisms exhibit complex behaviours, such as *Drosophila* courtship and learning (Giurfa, 2013; Chiang *et al*., 2011; Herculano-Houzel, 2009). Furthermore, many of the genes that influence behaviour in studies using genetic model organisms have homologues in other organisms with related functions in behaviour. For example, homologues of *per* have been found in other invertebrates with similar daily rhythms in expression, two mouse *per* homologues are capable of performing circadian rhythm functions in the fly, and variation in a human *per* homologue contributes to circadian rhythm variation in humans (Grabek & Chabot, 2012; Shigeyoshi *et al*., 2002; Toh *et al*., 2001). These instances exemplify the candidate gene approach to the study of behaviour genetics. This approach capitalizes on the evolutionary conservation of gene sequence and function across taxa, allowing researchers to hypothesize on the functions associated with genes not previously studied, but with characterized homologues in other organisms (Fitzpatrick *et al*., 2005). This approach has led to the identification of putative roles in food-related behaviours for *foraging* homologues of several species, including the *Apis mellifera* honey bee (Ben-Shahar *et al*., 2002), *C. elegans* nematode (Fujiwara *et al*., 2002), *Bombus terrestris* bumblebee (Tobback *et al*., 2011), *Vespula vulgaris* wasp (Tobback *et al*., 2008), and *Pheidole pallidula* ant (Lucas & Sokolowski, 2009). This widespread conservation of function was predicted by Fitzpatrick and Sokolowski (2004) based on the role of *foraging* in food-related behaviours in the model organism *D. melanogaster*, and the presence of *foraging* homologues in various distantly related taxa, from insects and fish to mammals, including humans. Similar cross-species comparisons have been employed
for the identification of genes influencing dispersal in *Melitaea cinxia* butterflies (Christoph *et al*., 2005), “crib-biting” in horses (Hemmann *et al*., 2014), aggression in species of the *Canidae* family (Szczerbal *et al*., 2007), and anxiety-like behaviour and aggression in humans (Kim *et al*., 2009; Soliman *et al*., 2011).

Advances in neurogenetics have also allowed for the investigation of neural circuits required for behaviour, a necessary step towards understanding how genes mediate behaviour. The *GAL4/UAS* expression system, one of the powerful tools employed on this front, allows for the targeted expression of genes, or even the ablation of cellular function, in specific neurons (Brand & Perrimons, 1993). Efforts to characterize neural circuits for behaviours have already been successful in identifying circuits for some simple reflexive behaviours in *Drosophila*, and continue through such efforts as to map more complex regions of the nervous system, like the mushroom body (Aso *et al*., 2009; Sokolowski, 2001; Trimarchi *et al*., 1999).

While the study of behavior genetics has traditionally focused on the contributions of genes, the influence of the environment has received increased attention over the last 40 to 50 years (Barsky, 2010). In particular, social environment has been the topic of several studies. In addition to inherently social behaviours, such as aggression and courtship, many behaviours not strictly considered social are also affected by social environment, such as sleep (Ganguly-Fitzgerald *et al*., 2006; Lee & Hall, 2000; Levine *et al*., 2002; Schneider *et al*., 2012a; Yurkovic *et al*., 2006). Furthermore, the effect of social environment is not always proportional to the degree of sociality of the species. For example, *D. melanogaster* is typically considered to be a solitary organism, but studies have shown that sleep and circadian rhythm in the fruit fly are highly dependent
on social environment (Bloch et al., 2013; Ganguly-Fitzgerald et al., 2006; Levine et al., 2002; Schneider et al., 2012a). However, very little is known about the mechanisms (neural circuitry, genes) mediating the effects of social cues on behaviour (Eban-Rothschild & Bloch, 2012).

Paralleling the rise of behaviour genetics, animal behaviour studies experienced a paradigm shift towards studying animal cognition, starting in the 1960s (Menzel & Fischer, 2011). Previously, the inner workings of behaviour in animals were largely attributed to systems analogous of stimulus-reflex. This period saw the recognition that this stimulus-reflex system was not sufficient to explain all animal behaviours (Staddon, 1981). Cognition can be broadly defined as neural processes used by animals to perceive the environment and produce an appropriate action. This includes perception, learning, memory, and decision making (Shettleworth, 2001).

Cognitive processes are important for many traits critical to fitness, including habitat choice, food choice, predator avoidance, mate choice, and social behaviours (Dukas, 2004; Dukas & Bernays, 2000; Gowaty et al., 2003; Morse & Stephens, 1996; Persons et al., 2002; Weidt et al., 2008). Natural heritable variation in cognitive traits has also been documented, making cognition a prime substrate for natural selection (Dukas, 2004; Greenwood et al., 2000; Parasuraman et al., 2002). However, the complexity of the mammalian nervous system can be an obstacle to in-depth study of cognition (Dukas, 2004). The use of simple model organisms, like Drosophila, with their relatively small nervous systems can provide insight into the genetic variation underlying natural variation in cognition (Sokolowski, 2010). Insects display many of the same cognitive processes present in mammals, including perception (Gibson et al., 2010), working
memory (Menzel, 2009), learning (Giurfa, 2013), attention (van Swinderen, 2011),
problem solving (Dussutour et al., 2009) and decision making (Naug & Arathi, 2007),
and the simplicity of genetic manipulations in Drosophila make it an ideal model
organism for studying the roles of genetics, environment, and neural circuitry in complex
behaviours, such as cognition (D’Mello & Franklin, 2011; Özkaya & Rosato, 2012).

Oviposition site selection (OSS) in the fruit fly D. melanogaster has been
proposed as a useful model for studying simple decision making (Joseph et al., 2009;
Yang et al., 2008). OSS, like other decision making processes, involves the perception of
the environment, such as the quality of potential oviposition sites, and the selection of an
appropriate behaviour, which can manifest as the choice of whether or not to lay an egg
or where to lay an egg. While OSS may appear relatively simple in limited experimental
contexts, it is influenced by a vast array of environmental factors, discussed below. A
study by Yang et al., (2008) found that D. melanogaster OSS behaviours could be broken
down into three distinct phases: search, egg deposition, and rest and clean. Fruit flies
were observed probing sites with multiple taste organs during the search phase before
each egg deposition. Wildtype oviposition site preferences were only observed in flies
with functioning sugar-sensing gustatory neurons. Taste has also been implicated in OSS
in a number of other studies on D. melanogaster and other invertebrates (Joseph et al.,
2009; Maher et al., 2006; Matsuo, 2012; Ryuda et al., 2013; van Loon, 1995; Wanner &
Robertson, 2008).

In addition to relatively simple indicators of site quality, OSS and other decision-
making behaviours in D. melanogaster are known to be influenced by more complex
environmental factors, such as social cues, availability of alternative sites, distance
between sites and the size of the area surrounding sites (Battesti et al., 2012; Mery et al., 2009; Sarin & Dukas, 2009; Schwartz et al., 2012; Sheeba et al., 1998; Tinette et al., 2004; Yang et al., 2008). Studies by Sarin and Dukas (2009) and Battesti et al. (2012) demonstrated that *D. melanogaster* females show increased oviposition site preference for food types that they experienced with other mated females. Tinette et al. (2004) and Mery et al. (2009) found similar effects of social cues on food choice and mate choice, respectively. Yang et al. (2008) found that *D. melanogaster* females preferred to oviposit on bitter-tasting media over sweet sucrose-containing media when given the choice, but laid approximately equal numbers of eggs when only given bitter media or sweet media. Sheeba and colleagues (1998) previously found similar differences between choice and no-choice oviposition experiments. Yang et al. (2008) also found that preference for bitter over sweet media decreased with increasing distance between sites. Schwartz et al. (2012) found that oviposition preferences for sucrose-containing media over non-nutritive media increased as the size of the behavioural arena increased. In addition to environmental factors, considerable heritable variation in oviposition preference has been documented (Barker, 1992; Joshi et al., 1997; Miller et al., 2011; Sheeba et al., 1999).

While OSS may appear relatively simple when studied in one experimental setting, comprehensive study of OSS in *D. melanogaster* will necessarily involve the investigation of a wide variety of factors.

The *foraging* gene (*for*) of *D. melanogaster* has two known naturally occurring alleles, called *for*<sup>R</sup> and *for*<sup>s</sup>. *for* encodes a cGMP-dependent protein kinase (PKG) and the *for*<sup>R</sup> allele confers higher PKG activity levels in adult heads than the *for*<sup>s</sup> allele (Osborne et al., 1997). Originally characterized for its role in larval foraging behaviour, allelic
variation of for has since been shown to be responsible for variation in several food-related traits, including adult foraging, food intake and homeostasis, and sucrose responsiveness (Kaun et al., 2007; Pereira & Sokolowski, 1993; Scheiner et al., 2004; Sokolowski, 1980). for also influences OSS. When given a choice of high-nutrient (high sucrose, high yeast) and low-nutrient (low sucrose, low yeast) media, groups of female flies homozygous for forR (rovers) show a surprising preference for ovipositing on low-nutrient media while groups of female flies homozygous for forS (sitters) prefer to oviposit on high-nutrient media. Transgenically increasing for-mRNA expression on a sitter genetic background, using the panneuronal expression driver elav-GAL4, confers rover-like oviposition site preferences (McConnell, 2011). The discovery of a role of for in OSS has provided a new avenue of study for simple decision-making in D. melanogaster.

Interestingly, for is known to play a role in response to many of the factors known to impact OSS and other food-related decision-making behaviours. Rovers and sitters differ in their responsiveness to gustatory stimulation by sucrose in proboscis extension assays (Scheiner et al. 2004, Belay et al. 2007). It is possible that this variation in sensitivity results in different behavioural responses to components of oviposition substrates and produces the observed differences in OSS. While responses of rovers and sitters to yeast odours have been studied, there is no such data for response to gustatory stimulation with yeast (Shaver et al., 1998). Yeast is the other variable component in the oviposition substrate, and a key component of the D. melanogaster diet (Becher et al., 2012). Another way to examine differential preferences for the yeast component of the food is to test rovers and sitters for differences in responsiveness to glycerol, a
component of yeast required for *D. melanogaster* feeding preferences for yeast (Wisotsky *et al.*, 2011). Furthermore, if oviposition preferences are mediated by variation in gustatory sensitivity, transgenic manipulations that change the expression of *for* in gustatory pathways, such as in sensory neurons or taste integration centers like the suboesophageal ganglion (SOG), may produce rover/sitter differences in OSS.

Interactions of *for* with the social environment may also impact OSS, as *for* is known to play a social role in *D. melanogaster* and other organisms; *for* modulates the effect of social environment on performance in cognitive tasks in *D. melanogaster*, and the *for* homologues of the honeybee, *A. mellifera*, and the ant, *P. pallidula*, are involved in social traits (Beh-Shahar *et al.*, 2002; Kohn *et al.*, 2013; Lucas & Sokolowski, 2009). The *A. mellifera* homologue of *for* plays a role in the switch from nurse to forager, impacting the social structure of the colony (Ben-Shahar *et al.*, 2002). The *P. pallidula* homologue of *for* plays a role in the transition of worker ants from defending behaviours to foraging behaviours in response to the needs of the colony (Lucas & Sokolowski, 2009).

With identified roles in OSS, and in factors influencing it, the *D. melanogaster for* gene presents an opportunity to investigate how genetic factors can produce variation in OSS. This is a process that is not only ecologically important for *D. melanogaster*, but can also serve as a model for understanding cognitive processes in other animals. In the present thesis, I address three questions: 1) Is the role of *for* in OSS affected by the availability of alternate oviposition site choices? 2) Is the role of *for* in OSS sensitive to the density of ovipositing females? 3) Is *for* expression in gustatory pathways, including
primary sensory neurons and higher-order structures, responsible for the observed rover/sitter differences in OSS?
Materials and Methods

(a) Fly strains and rearing conditions

The rover (+;for$^R$;+) and sitter (+;for$^S$;+) strains are described in Osborne et al. (1997). The laboratory-derived sitter mutant strain (+;for$^{s2}$;+) was generated on the rover genetic background and is homozygous for the for$^{s2}$ allele (de Belle et al., 1989, Pereira & Sokolowski 1993, de Belle et al., 1993). The for$^{s2}$ strain has sitter-like for-mRNA and for-PKG activity levels, and foraging behaviour (Osborne et al., 1997). The for$^{s2}$ strain is used as a control for the effects of genetic background. All three strains share a co-isogenic third chromosome from the for$^R$ strain.

All GAL4 and UAS transgenes used in this study were located on the third chromosome. The 129Y-GAL4 strain was obtained from the Bloomington Drosophila Stock Center. The ILP7-GAL4 strain was provided by Dr. Yuh Nung Jan (University of California, San Francisco). The Gr5a-GAL4 strain was provided by Dr. Michael Gordon (University of British Columbia). The Gr64e-GAL4 strain was provided by Dr. Anupama Dahanukar (University of California, Riverside). The UAS-forT1a strain was provided by Dr. Marla Sokolowski (University of Toronto). Each GAL4 and UAS transgene was crossed onto a sitter-derived first and second chromosome genetic background to produce +;for$^S$;129Y-GAL4, +;for$^S$;ILP7-GAL4, +;for$^S$;Gr5a-GAL4, +;for$^S$;Gr64e-GAL4, and +;for$^S$;UAS-forT1a.

Flies were maintained in 170 mL plastic bottles (VWR) on 40 mL of standard fly media, at 23 ± 1 °C and 65 ± 5 % relative humidity, on a 12L:12D light cycle, with lights off at 1900 hours. One litre of standard fly media contained 50 g of active dry yeast.
(Fleischmann’s), 100 g of sucrose, 17.43 g of agar, 0.1 g of KH₂PO₄, 8 g of C₄H₄KNaO₆, 0.5 g of NaCl, 0.5 g of MgCl₂, 0.5 g of Fe₂(SO₄)₃, and 5 mL of propionic acid.

(b) Fly collection and preparation

All adult flies were removed from fly stock bottles the day prior to collecting flies for experiments without anesthesia. Newly-eclosed flies were removed to 50 mL vials (VWR) on 10 mL of standard fly media daily, and maintained at rearing conditions.

For oviposition assays, flies were put in groups of 64 females and 20 males per 50 mL vial, under light CO₂ anesthesia. This 3:1 ratio of females to males was chosen to facilitate mating while limiting stress to females by males (McConnell, 2011). Males were removed 23-24 hours prior to experiments under light CO₂ anesthesia. For experiments where females were placed on a different substrate and female numbers were altered, these procedures were done at this time (see (f) Experiment 3). Females were assayed 3 days after collection.

For proboscis extension response (PER) assays, flies were placed in groups of 20 flies per 50 mL vial under light CO₂ anesthesia, with virgin females kept in same-sex vials. Flies were transferred to non-nutritive vials containing 5 mL of 1.74% agar without anesthesia 4 ± 0.5 hours prior to experiments in order to increase the responsiveness of flies in the assay (Scheiner et al., 2004). A longer food-deprivation period was not used since longer deprivation times have been shown to reduce the difference in foraging path length between rovers and sitters (Sokolowski & Riedl, 1999) and affect global gene expression levels of rovers and sitters differently (Kent et al., 2009). Flies were assayed 3 days after collection.
(c) Oviposition arena preparation

Oviposition arenas were constructed from translucent plastic containers (11 cm x 11 cm x 5.5 cm, Dollarama). A hole was cut into the lid to allow for gas exchange and transfer of flies. This hole was plugged with foam during experiments. Oviposition substrate was prepared in two types: high-nutrient and low-nutrient. Both substrate types were based on the standard rearing media, with modifications. The low-nutrient substrate had an 85% reduction in yeast and sucrose content. Both high- and low-nutrient substrates contained 2.6 g/L powdered charcoal to increase the visibility of eggs. Four sites of oviposition substrate were provided in 1.5 mL microcentrifuge tube caps (Axygen, VWR), filled to a highly convex surface, and fixed to the corners of a 4 cm by 4 cm unmarked square around the center of the arena with modeling putty. In arenas with both high- and low-nutrient substrate, the sites were differentiated by a mark of black permanent marker on the caps. Relative positions of the substrate types and markings were randomly assigned using a random number generator.

(d) Experiment 1: Rover and sitter OSS

Oviposition arenas were prepared as in section (c) above with two high-nutrient substrate sites and two low-nutrient substrate sites. Sixty-four rover, sitter, or sitter mutant females were assayed in each oviposition arena starting between 1530 and 1600 hours, and maintained at 25 ± 1 °C and 65 ± 5 % relative humidity overnight, on a 12L:12D light cycle, with lights off at 1900 hours. Flies were removed from the arenas
with CO₂ anesthesia between 0930 and 1000 hours the following day and the eggs on each site were counted.

(e) Experiment 2: No-choice oviposition

Each oviposition arena was prepared as in section (c) above with either four high-nutrient substrate sites, or four low-nutrient substrate sites. Sixty-four rover, sitter, or sitter mutant females were assayed in each oviposition arena starting between 1530 and 1600 hours, and maintained at 25 ± 1 °C and 65 ± 5 % relative humidity overnight, on a 12L:12D light cycle, with lights off at 1900 hours. Flies were removed from the arenas with CO₂ anesthesia between 0930 and 1000 hours the next morning and the eggs on each site were counted.

(f) Experiment 3: OSS in varying female density

Rovers, sitters, and sitter mutants were separated into testing groups of 4, 8, 16, 32, and 64 female flies, and were stored in 50 mL vials (VWR) on 5 mL of 1.74% agar at rearing conditions (to promote egg-laying in the assay) 23-24 hours prior to the assay. To limit possible confounding effects of starvation (Kent et al., 2009; Sokolowski & Riedl, 1999), a glass capillary tube (0.8-1.1 mm by 100 mm, Kimble Chase) filled with 10% sucrose solution was inserted through the side of the foam used to plug the vial. This procedure did not change strain differences in OSS, while simultaneously increasing egg-laying in the oviposition assay (see Appendix 1).

Oviposition arenas were prepared with two high-nutrient substrate sites and two low-nutrient substrate sites. Groups of 4, 8, 16, 32, or 64 female flies from the rover,
sitter, or sitter mutant strains were assayed in each oviposition arena starting between 1530 and 1600 hours, and maintained at 25 ± 1 °C and 65 ± 5 % relative humidity overnight, on a 12L:12D light cycle, with lights off at 1900 hours. Flies were removed from the arenas with CO₂ anesthesia between 0930 and 1000 hours the following day and the eggs on each site were counted.

(g) Experiment 4: Sucrose responsiveness

The PER assay was adapted from studies by Scheiner et al. (2004) and Shiraiwa and Carlson (2007) by Bryon Hughson, personal communication. Mated males, mated females, and virgin females from the rover, sitter, and sitter mutant strains were assayed. Single flies were isolated by aspirating by mouth. The fly was ejected via blowing and flicking into a 200 μL pipette tip cut to accommodate the head and one leg of an adult fly. Once the fly stopped moving in the pipette tip, the protruding tarsus was touched with a toothpick soaked in autoclaved distilled water. Flies that extended their proboscis in response to this water control were removed from the study to avoid assaying flies that would respond based on thirst (Scheiner et al. 2004). This accounted for less than 2 % of trials. Flies that did not respond to water were presented with six sucrose solutions in the same fashion, at concentrations of 0.1 %, 0.3 %, 1 %, 3 %, 10 %, and 30 % (w/v) in randomized order (Scheiner et al. 2004, Belay et al. 2007). A response was recorded if the fly extended its proboscis upon stimulation with the toothpick. The number of sucrose solutions that elicited a response represented the Response Score, giving a maximum score of 6.
(h) Experiment 5: Glycerol responsiveness

The PER assay was performed as in Experiment 4. Glycerol solutions were used in the place of sucrose solutions, at the same concentrations of 0.1 %, 0.3 %, 1 %, 3 %, 10 %, and 30 % (w/v).

(i) Experiment 6: Glycerol extraction and quantification

Autoclaved yeast suspensions were prepared and centrifuged at 13000 rpm for 3 minutes. Supernatant was removed for glycerol quantification. Glycerol concentrations were measured according to the manufacturer’s protocol for the Free Glycerol Reagent (Sigma-Aldrich, product no.: F6428).

(j) Experiment 7: Responsiveness to high- and low-nutrient glycerol concentrations

The PER assay was performed as in Experiment 4. Glycerol solutions were used in the place of sucrose solutions, at concentrations of 0.012 % and 0.0017 %. The proportion of flies responding to each solution was used instead of response scores.

(k) Experiment 8: OSS with manipulation of for expression

Expression of for was manipulated using the GAL4/UAS system. Experimental manipulations were generated by crossing females of the GAL4 strains to males of the UAS-forT1a strain. Negative GAL4 and UAS controls were generated by crossing females of the GAL4 strains and males of the UAS-forT1a strain to the sitter strain, respectively.
Oviposition arenas were prepared as in section (c) above, and the assay was performed as in Experiment 1, using the GAL4/UAS manipulation flies and their respective controls.

(I) Analyses

For oviposition assays, proportion and count data were transformed by arcsine-square-root, log, log(x + 1), or cube root transformation to ensure normality and homoscedasticity where possible. Normality and homoscedasticity were assessed with a Shapiro-Wilk test and Levene’s test, respectively. Where it was necessary to assess the effects of two independent variables (i.e. strain and substrate type, strain and fly density) a Two-Way ANOVA was used. The effects of strain and substrate type were assessed using a One-Way ANOVA for normal and homoscedastic data, a Welch’s F test for normal and heteroscedastic data, and a Wilcoxon Rank Sums test for non-normal data. Groups were compared with Tukey, Games-Howell, and Steel-Dwass post hoc tests, respectively. The effect of fly density was assessed using least squares linear regression.

For PER assays, response scores were analyzed with One-Way and Two-Way ANOVAs. Proportion data were analyzed using logistic regression.

Games-Howell post hoc tests were performed in IBM SPSS Statistics 22. All other statistical analyses were performed using JMP 11 (SAS Institute).
Results

Experiment 1: Rover and sitter OSS

Before investigating the mechanisms mediating the effect of for on OSS, I replicated previous findings (McConnell, 2011) by assaying oviposition site preferences of rovers, sitters, and sitter mutants for high-nutrient versus low-nutrient sites in groups of 64 females, over a period of 18 hours. As previously reported, rovers laid a significantly higher proportion of their eggs on low-nutrient substrate (67.78 ± 3.78 %, mean ± s.e.m., N = 20) than sitters (39.49 ± 4.56 %, N = 20) or sitter mutants (26.46 ± 4.25 %, N = 20) (F2,57 = 22.10, p < 0.0001, Tukey post hoc, Fig. 1), as well as significantly more eggs in total (43.05 ± 5.98 eggs) than sitters (14.15 ± 2.00 eggs) and sitter mutants (16.75 ± 1.39 eggs) (F2,33.37 = 14.54, p < 0.0001, Games-Howell post hoc, Fig. 2). In order to account for the potential confounding effects of gregarious oviposition, paired t-tests were used to compare the number of eggs laid on the two high-nutrient sites, and the two low-nutrient sites of each assay. All strains showed significant signs of aggregation across both substrate types. However, this comparison also showed that the three strains aggregated similarly. Rovers, sitters, and sitter mutants gave highly similar correlation coefficients for the number of eggs on the two high-nutrient sites (0.45, 0.48, 0.43, respectively) and two low-nutrient sites (0.72, 0.83, 0.76, respectively). Thus, variation in gregarious oviposition cannot explain the observed variation in substrate-type preference. Having confirmed previous findings on OSS in rovers and sitters, I moved on to investigate whether this rover/sitter variation in OSS is specific to choice situations.
**Figure 1: Oviposition assay.** Proportion of eggs laid on low-nutrient substrate by groups of 64 mated *D. melanogaster* females given a choice of high- and low-nutrient substrate.

Rovers laid a significantly higher proportion of their eggs on low-nutrient substrates compared to sitters and sitter mutants. N = 20 for each strain. Error bars represent the s.e.m.
**Figure 2: Oviposition assay.** Mean number of eggs laid by groups of 64 mated *D. melanogaster* females, given a choice of high- and low-nutrient substrate. The mean number of eggs laid by rovers was significantly higher than that of sitters and sitter mutants. N = 20 for each strain. Error bars represent the s.e.m.

**Experiment 2: No-choice oviposition**

I next asked whether rover/sitter variation in OSS was specific to choice situations or would be reflected in no-choice situations as well. I assayed the number of eggs laid by rovers, sitters, and sitter mutants in groups of 64 females in a no-choice environment where I provided either high- or low-nutrient substrate for oviposition sites, over a period of 18 hours. Assessing the roles of strain and substrate type on the total number of eggs laid, in a no-choice situation I found significant effects of substrate type (F_{1,114} = 4.14, p = 0.04) and strain (F_{2,114} = 82.46, p < 0.0001). I was unable to detect a significant
substrate × strain interaction (F_{2,114} = 1.02, p = 0.36). When I assessed the effect of food type on the number of eggs laid for each strain separately, however, I found no significant effect in rovers (F_{1,38} = 2.72, p = 0.11), sitters (F_{1,38} = 0.48, p = 0.50) or sitter mutants (F_{1,38} = 0.95, p = 0.34). In assays with high-nutrient sites, rovers laid significantly more eggs (149.20 ± 20.47 eggs, N = 20) than sitter mutants (45.50 ± 7.05 eggs, N = 20), and both strains laid significantly more eggs than sitters (14.05 ± 2.31 eggs, N = 20) (F_{2,36.04} = 39.41, p < 0.0001, Games-Howell post hoc, Fig. 3). In assays with low-nutrient sites, rovers again laid significantly more eggs (99.90 ± 15.30 eggs, N = 20) than sitter mutants (35.95 ± 3.69 eggs, N = 20), and both strains laid significantly more eggs than sitters (10.90 ± 1.44 eggs, N = 20) (F_{2,35.18} = 42.65, p < 0.0001, Games-Howell post hoc, Fig. 3).

The lack of a significant substrate × strain interaction term indicates that rover/sitter variation in site preference is specific to choice behaviours. Unlike in a choice environment, where groups of rover females laid the majority of their eggs on low-nutrient substrate (Experiment 1), these data suggest that, in a no-choice environment, rovers, sitters, and sitter mutants do not perceive a difference in attractiveness between the high- and low-nutrient substrates, or may perceive the high-nutrient substrate to be only weakly more attractive. I next asked whether this choice behaviour was sensitive to changes in social environment.
Figure 3: No-choice oviposition assay. Mean number of eggs laid by groups of 64 mated *D. melanogaster* females, given a single choice of either high- or low-nutrient substrate. Rovers laid significantly more eggs on average than sitters and sitter mutants, and sitter mutants laid significantly more eggs than sitters. Substrate type did not significantly affect the number of eggs laid for any of the individual strains tested, though analysis by two-way ANOVA for the effects of strain, substrate type, and the interaction did indicate an effect of substrate type. N = 20 for each strain, on each substrate type. Error bars represent the s.e.m.

Experiment 3: OSS in varying female density

To investigate the role of social environment in OSS in rovers and sitters, I manipulated the social environment in the oviposition assay by varying the density of mated females. I assayed oviposition site preferences of rovers, sitters, and sitter mutants in groups of 4, 8, 16, 32, and 64 females per arena with high- versus low-nutrient
substrates. Smaller groups were not assayed as they laid too few eggs to generate full datasets (see Appendix 2). Assessing the effects of strain and female density on the proportion of eggs laid on low-nutrient substrate sites, I found the overall model was significant ($F_{5,297} = 18.14, p < 0.0001$). There was a significant effect of strain ($F_{2,297} = 39.58, p < 0.0001$), density ($F_{1,297} = 4.55, p = 0.03$), and the strain $\times$ density interaction ($F_{2,297} = 3.40, p = 0.03$). In rovers, fly density explained a significant proportion of the variance in the proportion of eggs laid on low-nutrient substrate ($R^2 = 0.14, F_{1,98} = 16.13, p < 0.0001$), with the proportion of eggs on low-nutrient increasing with the number of females per arena ($b = 0.0037$). I was unable to detect a significant effect of female density in sitters ($R^2 = 0.003, F_{1,98} = 0.26, p = 0.61$) or sitter mutants ($R^2 < 0.0001, F_{1,101} = 0.01, p = 0.94$) (Fig. 4).
**Figure 4: Oviposition assay.** Proportion of eggs laid on low-nutrient substrate by groups of 4, 8, 16, 32, and 64 mated *D. melanogaster* females given a choice of high- and low-nutrient substrate. Rovers showed increasing preference for low-nutrient substrate as the number of mated females in the arena increased. Sitter and sitter mutant oviposition site preference were not significantly affected by the density of females. N = 23 for sitters, 4 females per arena. N = 20 for all other groups. Error bars represent the s.e.m.

**Experiment 4: Sucrose Responsiveness**

I next asked if gustatory neural circuitry mediates the role of *for* in OSS, first by investigating the role of *for* in gustatory sensitivity to the variable components of the oviposition substrates, sucrose and yeast. I first replicated previously reported findings of differences in responsiveness to sucrose in rovers and sitters (Scheiner et al. 2004, Belay et al. 2007). I tested mated males, mated females, and virgin females of the rover, sitter, and sitter mutant strains in the PER assay, using sucrose. Assessing the roles of strain,
sex and mating condition, and their interaction on sucrose response score, I found an overall significant model \((F_{8,144} = 2.60, p = 0.01)\) with a significant main effect of strain \((F_{2,144} = 6.48, p = 0.002)\). I was unable to detect an effect of sex and mating condition \((F_{2,144} = 1.35, p = 0.26)\) or strain \(\times\) sex and mating condition interaction \((F_{4,144} = 1.40, p = 0.24)\). Assessing strain alone, rover sucrose response scores \((0.86 \pm 0.12, N = 43)\) were significantly higher than those of sitters \((0.43 \pm 0.09, N = 75)\) and sitter mutants \((0.31 \pm 0.13, N = 35)\) \((F_{2,150} = 5.61, p = 0.004, \text{Tukey post hoc}, \text{Fig. 5})\).

**Figure 5: Proboscis extension with sucrose.** Sucrose response scores obtained from the PER assay with *D. melanogaster*. Rovers were significantly more responsive to stimulation with sucrose than sitters and sitter mutants. \(N = 43\) for rovers, \(N = 75\) for sitters, and \(N = 35\) for sitter mutants. Error bars represent the s.e.m.
Experiment 5: Glycerol Responsiveness

After confirming the role of for in responsiveness to sucrose, I investigated whether for also plays a role in responsiveness to yeast, via glycerol. Glycerol was used here because no significant strain effects were found in response to presentation with whole yeast (see Appendix 3). I tested mated males, mated females, and virgin females of the rover, sitter, and sitter mutant strains in the PER assay using glycerol. The main effects of strain, sex and mating condition, and their interaction on glycerol response scores showed an overall significant model ($F_{8,295} = 5.46, p < 0.0001$), with a significant main effect of strain ($F_{2,295} = 15.20, p < 0.0001$). I was unable to detect any significant effect of sex and mating condition ($F_{2,295} = 1.75, p = 0.18$) or the strain $\times$ sex and mating condition interaction ($F_{4,295} = 1.02, p = 0.40$). Excluding sex and mating condition from the analysis, rovers had significantly higher glycerol response scores ($0.78 \pm 0.07, N = 109$) than sitters ($0.31 \pm 0.09, N = 75$) and sitter mutants ($0.19 \pm 0.07, N = 120$) ($F_{2,301} = 17.88, p < 0.0001$, Tukey post hoc, Fig. 6).
Figure 6: Proboscis extension with glycerol. Glycerol response scores obtained from the PER assay with *D. melanogaster*. Rovers were significantly more responsive to stimulation with glycerol than sitters and sitter mutants. N = 109 for rovers, N = 75 for sitters, and N = 120 for sitter mutants. Error bars represent the s.e.m.

Experiment 6: Glycerol extraction and quantification

To determine how this tested range of glycerol concentrations compared to glycerol concentrations found in the yeast used in the oviposition assays, I extracted glycerol from suspensions of yeast at the same concentrations used in high- and low-nutrient oviposition substrates (McConnell, 2011), and measured the glycerol concentrations using Free Glycerol Reagent. The high yeast concentration had 0.012 % (w/v) glycerol and the low yeast concentration had 0.0017 % (w/v) glycerol. These
concentrations lie well below the range of glycerol concentrations tests shown in Figure 6 above.

**Experiment 7: Responsiveness to high- and low-nutrient glycerol concentrations**

In order to test whether the role of for in responsiveness to glycerol extends to concentrations found in the oviposition substrates, I again tested mated males, mated females, and virgin females of the rover, sitter, and sitter mutant strains using the PER assay. In this experiment, flies were only exposed to 0.012% and 0.0017% glycerol, to reflect conditions more representative of oviposition substrates. Since I only tested two concentrations instead of six (as in experiments 4 & 5) and the response to each concentration was of particular interest, I did not use the response score metric to assess responsiveness, and instead looked at the proportion of tested flies that responded to each concentration. I first assessed the effects of strain (rover, sitter, sitter mutant) and sex and mating condition (mated male, mated female, virgin female) on the number of flies exhibiting PER to 0.012% and 0.0017% glycerol. Analysis of responses to the high concentration showed an overall significant model ($\chi^2 = 16.89$, DF = 8, $p = 0.03$), with a significant effect of strain ($\chi^2 = 10.31$, DF = 2, $p = 0.006$). I was unable to detect a significant effect of sex and mating condition ($\chi^2 < 0.0001$, DF = 2, $p = 1.00$) or strain $\times$ sex and mating condition interaction ($\chi^2 = 3.41$, DF = 4, $p = 0.49$). Analysis of responses to the low concentration showed no significance ($\chi^2 = 12.53$, DF = 8, $p = 0.13$). I was unable to detect any significant effects of strain ($\chi^2 < 0.0001$, DF = 2, $p = 1.00$), sex and mating condition ($\chi^2 < 0.0001$, DF = 2, $p = 1.00$) or strain $\times$ sex and mating condition interaction ($\chi^2 = 5.23$, DF = 4, $p = 0.26$). With no effect of sex and mating condition or
the interaction at either glycerol concentration, I pooled the data from the different sex and mating condition groups and looked at the main effect of strain. I found a significant effect of strain on response to the high concentration \( (\chi^2 = 12.24, \text{DF} = 2, p = 0.002) \) but no significant effect on response to the low concentration \( (\chi^2 = 1.14, \text{DF} = 2, p = 0.57) \). Less than 5% of rovers (3 of 69 flies), sitters (2 of 71), and sitter mutants (1 of 71) responded to the low glycerol concentration. Rovers alone showed an increased response to the high glycerol concentration, with over 10% responding (7 of 69) compared to less than 2% of sitters (1 of 71) and sitter mutants (0 of 71) responding (Fig. 7). With evidence for strain differences in responsiveness to tastants for both of the variable components in the oviposition substrates (sucrose and yeast), I next asked if differential for expression in gustatory neural circuitry was sufficient to confer differences in OSS.
Figure 7: Proboscis extension with high and low glycerol. Proportion of *D. melanogaster* flies responding to glycerol concentrations representative of low-nutrient substrate (0.0017 %) and high-nutrient substrate (0.012 %) in the PER assay. The proportions are used here in place of the response scores. Rovers, sitters, and sitter mutants did not vary in response to the low glycerol, but significantly varied in response to the high glycerol. N = 69 for rovers. N = 71 for sitters and sitter mutants. I was unable to include error bars, as each group is represented by a single proportion.

Experiment 8: OSS with manipulation of *for* expression

Upregulation of *for*-mRNA expression throughout the nervous system using the panneuronal expression driver *elav-GAL4* confers rover-like oviposition site preferences on a sitter genetic background (McConnell, 2011). With identified roles in feeding response to sucrose and glycerol, I investigated whether elevated *for* expression in
gustatory neurons sensitive to these tastants could contribute to the greater preference of rover females for low-nutrient oviposition substrate compared to sitter females. I employed the GAL4/UAS system to drive for-mRNA expression in gustatory neurons sensitive to sucrose and glycerol on a sitter genetic background, using a Gr5a-GAL4 strain and a Gr64e-GAL4 strain, respectively. I also looked at possible roles of for in higher-order neural structures involved in taste and oviposition using a 129Y-GAL4 strain and an ILP7-GAL4 strain, which express in the suboesophageal ganglion and antennal nerve (Joiner et al., 2006), and suboesophageal ganglion, thoracico-abdominal ganglion, and female reproductive system (Yang et al., 2008), respectively. GAL4/UAS manipulations with these strains were tested alongside the relevant controls, in groups of 64 females in two-choice oviposition assays with high- and low-nutrient oviposition substrates. For each GAL4 driver, I assessed the main effect of treatment (UAS control, GAL4 control, GAL4/UAS treatment) on the proportion of eggs laid on low-nutrient substrate. As the different GAL4 drivers were tested simultaneously, the same UAS control is used across all four GAL4 drivers.

The GAL4/UAS treatment did not lay a significantly higher proportion of eggs on the low-nutrient treatment than both relevant controls for any of the four tested GAL4 drivers (Fig. 8). I was unable to detect a significant effect of treatment for the Gr64e-GAL4 ($\chi^2 = 4.27, \text{DF} = 2, p = 0.12$) and ILP7-GAL4 drivers ($F_{2,57} = 0.97, p = 0.39$). For the Gr5a-GAL4 driver, the GAL4 control laid a significantly higher proportion on the low-nutrient substrate ($41.20 \pm 4.19\%$, $N = 20$) than the GAL4/UAS treatment ($62.04 \pm 3.91\%$, $N = 20$) and the UAS control ($44.91 \pm 6.44\%$, $N = 20$, $F_{2,36.78} = 7.04, p = 0.003$, Games-Howell post hoc). For the 129Y-GAL4 driver, the GAL4/UAS treatment laid a
significantly lower proportion on the low-nutrient substrate (31.39 ± 6.68 %, N = 20) than the GAL4 control (64.04 ± 5.95 %, N = 20, F_{2,57} = 6.83, p = 0.002, Tukey post hoc).

**Figure 8: Oviposition assay with transgenic for-mRNA upregulation.** Proportion of eggs laid on low-nutrient substrate by groups of 64 mated females given a choice of high- and low-nutrient substrate. The GAL4/UAS system was used to upregulate for-mRNA expression with *Gr5a-GAL4, Gr64e-GAL4, 129Y-GAL4*, and *ILP7-GAL4* expression drivers on a sitter genetic background. Each GAL4/UAS manipulation failed to increase oviposition preference for low-nutrient substrate compared to the UAS-for control and relevant GAL4 control. N = 20 for all treatments. Error bars represent the s.e.m.
Discussion

OSS in *D. melanogaster* has been proposed as a useful model for studying simple decision-making processes (Joseph *et al.*, 2009; Yang *et al.*, 2008). Relatively little is known about this process, but the *for* gene, which is known to play a role in variation in OSS, provides a new avenue for studying how natural genetic variation interacts with the environment and neural circuitry to influence OSS. In this thesis, I replicated previously reported findings on OSS differences among strains of the *for* gene, provided evidence for the influence of choice versus no-choice environments and social environment on these strain differences, and replicated previous findings and provided new evidence of strain differences in gustatory response to sucrose and glycerol, respectively. I also began to investigate the role of *for* expression in specific neural circuitry for OSS. These findings are discussed further below.

Rover/sitter differences in OSS are specific to choice environments

As previously reported, the *for*<sup>R</sup> allele conferred oviposition site preference for low-nutrient (low sucrose, low yeast) over high-nutrient (high sucrose, high yeast) substrate in groups of rovers, while the *for*<sup>s</sup> and *for*<sup>s2</sup> alleles conferred preference for high-nutrient substrate in groups of sitters and sitter mutants, respectively (Fig. 1). Furthermore, while *D. melanogaster* females are known to aggregate in oviposition, this behavioural tendency alone is insufficient to explain the observed differences in site preference (Ruiz-Debreuil & Köhler, 1994). Comparison of the number of eggs laid on each of the two high-nutrient sites, as well as each of the two low-nutrient sites in each assay with paired t-tests did show an effect of aggregation in all strains and substrate
types. However, highly similar correlation coefficients across all strains for each substrate type revealed that gregarious oviposition could not explain the observed substrate type preferences.

These strain differences in site preference were no longer apparent when tested in no-choice environments. When given only high- or low-nutrient sites, rovers, sitters, and sitter mutants each laid a similar number of eggs per arena on the two substrate types, though there was a non-significant trend across all strains to oviposit more in high-nutrient environments (see Experiment 2, Fig. 3). The ability of *D. melanogaster* females to withhold their eggs when appropriate sites are not available is well-documented (Allemand & Boulétreau-Merle, 1989; Eisses, 1997; van Delden & Kamping, 1990). If rover/sitter differences in site preference were a product of differences in inherent attractiveness of the two substrates, rovers would be expected to lay more eggs in a low-nutrient environment, and sitters and sitter mutants more in a high-nutrient environment. The data instead suggest that more complex mechanisms may underlie rover/sitter differences in OSS. The role of spatially separated sites in determining site preference may indicate the involvement of memory and/or multimodal sensory integration (e.g. taste and smell) in this variation, such that oviposition decisions made at one site are done so with information on other available sites (Miller et al., 2011). There are precedents for the involvement of these processes. Multimodal sensory integration in *D. melanogaster* has been studied in the context of courtship and search behaviours (Frye & Dickinson, 2003; Griffith & Ejima, 2009). *D. melanogaster* females are also known to use distinct sets of information about their environment during OSS, such as site nutrition and container size (Schwartz et al., 2012). Several studies have also demonstrated a role of
memory in *D. melanogaster* OSS (Battesti *et al*., 2012; Cadieu *et al*., 2000; Kawecki & Mery, 2006; Mery *et al*., 2007; Sarin & Dukas, 2009). The implications for learning and memory are discussed further below.

One outstanding question is whether individuals interact with all available sites during this behaviour. A study by Yang *et al*. (2008) indicated that ovipositing females visit available sites in a search phase that occurs before each egg deposition. There is, however, evidence that perhaps not all individuals probe the available sites. A study by del Solar and Ruiz (1992) showed that flies selected for gregarious oviposition laid all of their eggs on one out of six nutritionally identical sites, compared to flies selected for low aggregation that spread out their eggs over all six sites. A study of food choice behaviour by Tinette *et al*. (2004) found that only a small subset of groups of flies probed the available sites, before the group settled on a preferred site. This group organization may be of particular relevance to the present findings, as the group impacts rover/sitter oviposition as well, discussed further below.

These findings further demonstrate a role of *for* specifically in decision-making, a process for which the availability of alternative options is central. Flexibility in relation to environmental cues is also a central component of this process (Schwartz *et al*., 2012). As such, the finding that rovers and sitters differ in flexibility of OSS in response to female density also contributes to the identification of a role of *for* in OSS as a decision-making process.
Rover/sitter differences in OSS are sensitive to female density

In addition to choice versus no-choice environments, my data suggests a role of social environment in rover/sitter differences in OSS (Experiment 3). Changes in the number of females per oviposition arena did not impact OSS in groups of sitters or sitter mutants, but did impact OSS in groups of rovers, with rovers showing an increasing preference for low-nutrient sites as the number of females increased (Fig. 4). The effect of fly density in rovers was likely not due to changes in the number of eggs laid on the substrates; pre-seeding oviposition sites with eggs has previously been shown not to influence rover/sitter site preferences, and increasing pan-neuronal for-mRNA expression with elav-GAL4 has also been shown to confer rover-like site preferences on a sitter genetic background without increasing the number of eggs laid relative to controls (McConnell, 2011). These findings fit into a broader paradigm of social environment influencing a variety of choice behaviours in D. melanogaster.

The strain-specific effect of social environment is consistent with differences in gregarious tendencies of the rover and sitter strains. Sitters are generally more gregarious at food sites than rovers, and thus may not experience much variation in local fly density between different female density treatments (Kohn et al., 2013). The local social environment experienced by the less gregarious rovers likely varies more widely with changes in fly density. If this is the case, the social environment experienced by rovers and sitters should be most similar in high-density experiments, where site preferences are also most divergent. Differences in gregarious behaviour alone could thus not explain rover/sitter differences in OSS in this case.
The *for* gene has been implicated in variation in adult learning and memory, a cognitive trait with a large social component and potential to impact OSS in *D. melanogaster* (Battesti *et al.*, 2012; Chabaud *et al.*, 2009; Foucaud *et al.*, 2013; Kohn *et al.*, 2013; Kuntz *et al.*, 2012; Mery *et al.*, 2007; 2009; Reaume *et al.*, 2010; Sarin & Dukas, 2009; Schneider *et al.*, 2012a; Wang *et al.*, 2008). When assayed for olfactory learning, rovers showed better short-term memory while sitters showed better long-term memory (Mery *et al.*, 2007). Rovers also showed a bias towards relying more on recently learned information over older information, which sitters did not (Reaume *et al.*, 2010). Rovers have also been found to have better short-term visual memory than sitters (Kuntz *et al.*, 2012; Wang *et al.*, 2008). It should be noted that previous studies on the role of *for* in learning and memory have shown an opposing trend in the interaction of *for* and social environment; learning in rovers was not sensitive to the presence of other rovers, while sitters showed higher learning ability in groups than in isolation (Foucaud *et al.*, 2013; Kohn *et al.*, 2013). Though I was unable to generate a full dataset for oviposition in single flies, single sitter females did not appear to show different site preferences to groups of sitter females (Appendix 2). Furthermore, unlike the present work, studies demonstrating the impact of learning and memory on OSS have necessarily involved the use of a training paradigm, or demonstrator individuals (Battesti *et al.*, 2012; Kohn *et al.*, 2013; Sarin & Dukas, 2009). Thus, it is not unlikely that the effect of social environment on OSS shown here is mediated by different mechanisms to those mediating its effect on learning and memory in rovers and sitters. The experiments described in this thesis were also not designed to quantify learning and memory. Future study of the mechanisms of variation in OSS would benefit from a deeper consideration of this process.
A possible alternative explanation for the role of social environment in OSS is the occurrence of female-female aggression. While most studies of aggression in *D. melanogaster* have focused on male-male interactions, a handful of studies have characterized female-female aggression (Nilsen *et al.*, 2004; Ueda & Kidokoro, 2002). A direct link between aggression and oviposition has not been demonstrated in *D. melanogaster*, though it has been suggested (Ueda & Kidokoro, 2002). A more direct connection between female-female aggression and oviposition has been made in the tephritid fruit fly genus, *Bactrocera*. In *Bactrocera tryoni* and *dorsalis*, females have been observed to interrupt oviposition to engage in aggressive interactions with other females (Pritchard, 1969; Shelly, 1999). In *B. dorsalis*, females that were observed ovipositing on a food source were also observed to engage in aggressive interactions on that food source more frequently than females that laid no eggs. Females that oviposited on a food source were also more likely to leave that food source because of a loss in an aggressive interaction compared to females not engaged in oviposition, which often left without provocation. Furthermore, changing the attractiveness of the food as an oviposition site concomitantly changed the amount of time spent ovipositing by females, and the incidence of female-female aggression on the food (Shelly, 1999). Thus, a clear link between female aggression and oviposition exists in *Bactrocera*. While no studies in *D. melanogaster* have specifically addressed this potential link, there is evidence to support it. Just as *B. dorsalis* females exhibited more aggression on food that was more attractive for oviposition, levels of female-female aggression in *D. melanogaster* are higher on food with live yeast colonies than on food without (Ueda & Kidokoro, 2002).
Fights among mated females also last longer and more readily increase in intensity than fights among virgin females (Nilsen et al., 2004).

Obviously, the occurrence of female-female aggression alone cannot explain the strain-specific effect of female density shown here. A role of for in aggression has not been described in the literature. One possibility is that the greater number of eggs laid by rovers compared to sitters (Fig. 2) may correlate with more time spent by rovers in oviposition behaviours. Just as more aggression was observed in ovipositing B. dorsalis females, this may lead to increased aggression in rovers. At higher female densities, such interactions may divert females from attractive high-nutrient sites to low-nutrient sites, while also serving to interrupt oviposition behaviours on high-nutrient sites. However, sitter and sitter mutant preference for high-nutrient sites was unchanged by prior substrate deprivation, despite laying more eggs than non-deprived rovers (see Appendix 1). Differences between rovers and sitters in memory may also contribute to differences in response to aggression or other social interactions. Rovers, with better short-term memory than sitters, may modulate their behaviour more readily based on previous interactions with other females.

Emergent group-level phenotypes such as this suggest the existence of dynamic group organization (Schneider et al., 2012a). Accordingly, D. melanogaster are known to have intricate social structures. In the case of D. melanogaster, these have been measured as Social Interaction Networks (SINs), which describe the frequency, interacting partners, and quality of social interactions satisfying certain criteria for relative positioning and duration (Schneider et al., 2012a; 2012b). Both males and females are known to form nonrandom SINs, though there are structural differences between them (Schneider et al.,
2012b). These are likely due to the differences in social behaviours between males and females. Aggression between males and females is quite different, for example. While aggressive interactions among males form social hierarchies, aggression in females does not (Nilsen et al., 2004). It is not yet clear how for might influence SINs, but genetic components to SIN structure have been identified in D. melanogaster (Schneider et al., 2012a; 2012b).

The findings presented here will likely have implications for natural populations, as D. melanogaster aggregate at food sites and peak in oviposition activity at dusk, thus exposing females to these complex and potentially dense social environments during oviposition (Allemand & Boulètreau-Merle, 1989; Gruwez et al., 1971; Schneider et al 2012a; Schwartz et al., 2012; Wertheim et al 2005). The strain-specific effect of density on OSS behaviours shown here will likely impact local larval densities and genotypic frequencies. The low mobility of larval D. melanogaster stops them from freely choosing their local environment, so the ovipositing pattern of adults is an important determining factor in early-life experience. This is of particular relevance to for, as density-dependent and negative frequency-dependent selection are known to act on for at the larval stage (Fitzpatrick et al., 2007; Sokolowski et al., 1997).

In addition to the ecology and evolution of D. melanogaster, further study into this system can elucidate the processes in individuals that are responsible for variation in these behaviours. Identification of key behaviours and social interactions to variation in OSS will further our knowledge of sensory pathways that individuals use to gather relevant environmental cues, higher-order structures that integrate this sensory information, and output circuitry for OSS behaviours. I discuss this further below.
Lastly, I investigated the role of gustatory pathways in rover/sitter differences in OSS, first by investigating the role of \textit{for} in responsiveness to gustatory stimulation with the variable components of the oviposition substrate. My data confirms previously reported \textit{for}-dependent variation in sucrose responsiveness, with rovers being more responsive than sitters and sitter mutants (Scheiner \textit{et al.} 2004, Belay \textit{et al.} 2007; Fig. 5). I also demonstrate \textit{for}-dependent variation in glycerol responsiveness for the first time, with rovers again showing higher responsiveness than sitters and sitter mutants (Fig. 6). Additionally, this difference in responsiveness extends to the low concentrations of glycerol found in the oviposition substrates, with rovers alone displaying an increase in response to the high glycerol concentration (Fig. 7). This may be indicative of a lower glycerol detection threshold in rovers.

Outside of the decision-making paradigm, this lower threshold of adult rovers would likely be useful in identifying sites of early yeast colonization, such as fruit that is at the very beginning stages of decomposition. This threshold lies well below the previously reported threshold for \textit{D. melanogaster} of 15 mM (0.14 \% w/v, compared to 0.012 \%) (Koseki \textit{et al.}, 2004). While such sites would have limited nutrition at the time of oviposition, they would also provide an opportunity to reduce intraspecific competition for resources since sitters would not make use of these sites until yeast colonization had progressed further. A recent study on \textit{D. melanogaster} dispersal by Edelsparre \textit{et al.} (2014) showed that adult rovers have greater dispersal tendencies, and disperse farther, than sitters. This would mean that, in addition to being more sensitive to sites of early
yeast colonization, rovers likely encounter these sites more often than sitters, making rovers well-adapted to finding and using new and previously unused food patches. Together with aggregative tendencies and the propensity of adults to spread yeast to new substrates, rovers may help to facilitate the movement and spread of *D. melanogaster* populations to new food patches (Stamps et al., 2012; Wertheim et al., 2002). Further work examining rover and sitter interactions with sites of early yeast colonization will be required before we can make more conclusive statements about the effects of gustatory sensitivity on the movement ecology of rovers and sitters.

The glycerol gustatory receptor, Gr64e, is expressed in two subsets of neurons: Gr5a- and E409-labeled neurons (Wisotsky et al., 2011). Gr5a is a gustatory receptor required for sugar detection, and is coexpressed with other gustatory receptors required for detection of various sugars (Montell, 2009). This raises the possibility that crosstalk between Gr64e and these other sugar receptors may contribute to yeast detection. This may explain why sitters and sitter mutants show no concentration-dependent response to gustatory stimulation with the high and low glycerol concentrations (see Experiment 7, Fig. 7), and show oviposition site preferences between sites varying only in yeast content when all sites contain high sucrose concentrations, but not low (Chen and Fitzpatrick, in prep).

Expression of the *C. elegans* for homolog, *egl-4*, in primary sensory neurons has been previously shown to influence behaviour (L'Etoile et al., 2002). I next investigated whether *for* expression similarly played a role in OSS via expression in primary gustatory neurons. Examining the role of these gustatory pathways in the setting of the oviposition assay, upregulating *for*-mRNA expression in sugar- and glycerol-sensing gustatory
neurons did not confer rover-like site preferences. I next looked at a second-order structure in the fruit fly gustatory pathway, the SOG. The SOG is commonly considered to be one of the primary taste-processing centers of the fruit fly brain; all labellar and some tarsal gustatory receptor neurons project primarily to the SOG (Mitchell et al., 1999; Thorne et al., 2004). Similar upregulation in the SOG using 129Y-GAL4 and ILP7-GAL4 also failed to confer rover-like site preferences.

These findings are not definitive in excluding these or other taste-related structures from further consideration; manipulations of for-mRNA expression were at the mercy of GAL4 expression levels, as well as expression patterns within the SOG. However, together with other results discussed above, they indicate that variation in gustatory perception of oviposition substrates may not be central to rover/sitter differences in OSS. Instead, for may be acting on OSS through circuitry that functions in social interactions such as aggression, learning and memory, or other structures central to decision-making. Disruption of gustatory and olfactory, but not visual or auditory, pathways has been shown to result in significantly altered SINs in D. melanogaster, and so may still hold some relevance to rover/sitter differences in OSS (Schneider et al., 2012a; 2012b). Olfactory cues have been shown to be an important mediator of social effects on circadian rhythm in D. melanogaster, a behavioural trait that, like OSS, may appear to be asocial on its face (Levine et al., 2002; Lone & Sharma, 2011). Study of circuitry for biogenic amines, such as dopamine, octopamine and serotonin, may also prove worthwhile, as these systems are known to modulate aggression and other social behaviours in D. melanogaster (Alekseyenko et al., 2010; Baier et al., 2002; Certel et al., 2010; Luo et al., 2014; Potter & Luo, 2008; Zhou et al., 2008). Several neural structures
are known to function in learning and memory, including the fan-shaped body, ellipsoid body, median bundle, and mushroom bodies (Liu et al., 2006; Pan et al., 2009; Pascual & Preat, 2001; Strausfeld et al., 2009; Zars et al., 2000). *for* expression in the ellipsoid body and the fan-shaped body is known to play a role in visual memory (Kuntz et al., 2012; Wang et al., 2008). The mushroom bodies may also hold particular promise. In addition to learning and memory, the mushroom bodies have been implicated in sensory integration, social interactions including aggression, decision-making, and oviposition (Brembs, 2009; Certel et al., 2010; Fleischmann et al., 2001; Joseph et al., 2009; Joseph & Heberlein, 2012; Wu & Guo, 2011; Xi et al., 2008; Zhang et al., 2007). In two-choice oviposition assays, loss of mushroom body function was found to abolish oviposition site preference, suggesting an important role in decision-making during oviposition (Joseph et al., 2009; Joseph & Heberlein, 2012). Furthermore, recent work on the honey bee *A. mellifera* has revealed a concentration of *for* homologue expression in the mushroom bodies (Thamm & Scheiner, 2014). The mushroom bodies and other higher-order structures will be prime candidates for further study of the role of *for* in OSS.

**Future work**

While I have demonstrated roles for choice and female density in rover/sitter differences in OSS, a number of outstanding questions remain as obstacles to identifying the mechanisms through which *for* acts on OSS. Many of these questions may be answered through direct observation of rovers and sitters during OSS behaviours, as opposed to the end-point measurement of number of eggs laid used in this study. For example, it is currently unknown if each female visits all available sites before making an
oviposition decision. Observation of individual movement during the oviposition assay will afford valuable insight on the sensory modalities that females may be using to assess site quality. Similarly, observations of the social cues and interactions that differ between groups of rovers and sitters will allow us to identify the mechanism by which female density influences OSS. Assaying oviposition of rovers in groups of sitters, and vice versa, will also be informative for whether rovers and sitters create a different social environment, or respond differently to social environment. Assaying mixes of the strains will also be necessary if this system is to be studied in the context of ecology and evolution. In natural populations of D. melanogaster, rovers and sitters will not be found in mutual isolation, and mixing strains in groups has been shown to uncover new behavioural responses not present in homogeneous groups (Kent et al., 2008; Krupp et al., 2008; Levine et al., 2002; Schneider, 2012a). Along these lines, the introduction of males into the system may also be informative, as this will add courtship and mating behaviours to the social environment and alter SIN structure, as would likely be experienced in natural populations.

Given the specificity to choice environments and the role of social environment, systems-level investigations of for and OSS will likely benefit from consideration of the mushroom bodies. Several GAL4 lines with expression in the mushroom bodies are available (Aso et al., 2009). While I used the GAL4/UAS system to increase for-mRNA expression, the use of a for-RNAi line may be more appropriate, as this will only influence for expression in regions where it is already expressed. Further investigation of the gustatory and olfactory systems will also be informative on the sensory modalities informing OSS decisions in rovers and sitters. Both senses are known to impact social
interactions as well as evaluation of oviposition site nutritional quality (Matsuo, 2012; Schneider et al., 2012a; 2012b). Selective ablation of these systems may help to reveal their relative contributions to OSS in rovers and sitters.
Conclusions

In this study, I identified, for the first time, clear roles of choice and female density in OSS by rovers and sitters. Rover/sitter differences in preference for high-versus low-nutrient sites were not reflected in oviposition patterns in no-choice environments. When assayed in a range of female densities, rovers alone showed a response in site preference to female density. These findings suggest a central role of for in OSS decision-making and social interactions. This study should prove highly informative for further research into the precise neural and social mechanisms mediating the influence of for on OSS. As a model of simple decision-making processes, such research will be instrumental in understanding the circuitry underlying cognitive processes, and how natural genetic variation can give rise to variation in these highly complex systems. This will have implications not only for studies in D. melanogaster and other insects, but also a wide variety of taxa with larger nervous systems that pose barriers to the study of cognition, such as in mammals.
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Appendices

Appendix 1. Substrate deprivation and OSS

Before testing oviposition preferences in varying fly densities, it was necessary to verify that the number of eggs laid could be increased by prior oviposition site deprivation, without altering site preferences, so that preferences could be assayed in small fly numbers. Flies of the rover, sitter, and sitter mutant strains were prepared as in Materials and Methods section (b), and deprived of nutritive substrate for oviposition and assayed as in section (f), only in groups of 64 mated females.

Rovers laid a significantly higher proportion of their eggs on the low-nutrient substrate (60.88 ± 3.91 %, N = 25) than sitters (25.69 ± 2.85 %, N = 20) and sitter mutants (36.67 ± 5.25 %, N = 15) (F2,57 = 21.96, p < 0.0001, Tukey post hoc, Fig. A1). Rovers also laid significantly more eggs on average (168.92 ± 14.65 eggs) than sitters (63.70 ± 7.55 eggs) and sitter mutants (66.67 ± 7.37 eggs) (F2,57 = 27.77, p < 0.0001, Tukey post hoc, Fig. A2). These significance relations are identical to those found in non-deprived flies (Experiment 1), indicating that the method I used for substrate deprivation does not impact strain differences in OSS.

While maintaining differences in OSS, substrate deprivation led to an increase in the number of eggs laid by approximately four times in each strain (168.92 ± 14.65 versus 43.05 ± 5.98 in rovers, 63.70 ± 7.55 versus 14.15 ± 2.00 in sitters, 66.67 ± 7.37 versus 16.75 ± 1.39 in sitter mutants).
Appendix 2. OSS in single flies

Initially, tests for the effect of female density on OSS (Experiment 3) were planned to include data on OSS with single mated females, in addition to the groups of 4, 8, 16, 32, and 64 mated females, shown in Fig. 4. However, single females laid too few eggs to obtain full datasets. Flies of the rover, sitter, and sitter mutant strains were prepared as in Materials and Methods section (b), and deprived of nutritive substrate for oviposition and assayed as in section (f), only with single females instead of groups. Out of the 31 rovers tested, 22 laid eggs during the assay, while only 7 out of 27 sitters and 2 out of 20 sitter mutants laid eggs during the assay. Analysis by Wilcoxon Rank Sums showed no effect of strain on the proportion of eggs laid on low-nutrient media ($\chi^2 = 2.70$, DF = 2, $p = 0.26$). On average, rovers, sitters, and sitter mutants each laid 28.26 ± 7.22, 21.43 ± 0.15, and 66.67 ± 33.33 % of their eggs on low-nutrient substrate, respectively.
Appendix 3. PER with yeast

To investigate the responsiveness of rovers and sitters to yeast, whole yeast was also used in the PER assay. Mated females, virgin females and mated males of the rover, sitter, and sitter mutant strains were prepared as in Materials and Methods section (b), and the assay was performed as in section (g), but with autoclaved yeast suspensions instead of sucrose. The same range of concentrations was used as with sucrose.

Assessing for the effects of strain, sex and mating condition, and their interaction on the yeast response score gave an overall insignificant model ($F_{8,168} = 1.81$, $p = 0.08$). I was unable to detect a significant effect of strain ($F_{2,168} = 2.09$, $p = 0.13$), sex and mating condition ($F_{2,168} = 3.04$, $p = 0.051$), or strain $\times$ sex and mating condition interaction ($F_{4,168} = 1.05$, $p = 0.38$). Rovers produced an average yeast response score of $0.21 \pm 0.08$ ($N = 58$), sitters $0.03 \pm 0.03$ ($N = 58$), and sitter mutants $0.18 \pm 0.07$ ($N = 61$).