1 **Correlated decision making across multiple phases of olfactory guided search in Drosophila**

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7

8 **Abstract**

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10 All motile organisms must search for food, often requiring the exploration of heterogeneous
11 environments across a wide range of spatial scales. Recent field and laboratory experiments with the
12 fruit fly, *Drosophila*, have revealed that they employ different strategies across these regimes, including
13 kilometer scale straight-path flights between resource clusters, zig-zagging trajectories to follow odor
14 plumes, and local search on foot after landing. However, little is known about the extent to which
15 experiences in one regime might influence decisions in another. To determine how a flies' odor plume
16 tracking during flight is related to their behavior after landing, I tracked the behavior of individually
17 labelled fruit flies as they explored an array of three odor emitting, but food-barren, objects. The
18 distance flies travelled on the objects in search of food was correlated with the time elapsed between
19 their visits, suggesting that their in-flight plume tracking and on-foot local search behaviors are
20 interconnected through a lossy memory-like process.

21

22 **Introduction**

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24 All moving organisms spend a significant amount of their time and energy searching, be it for food,
25 mates, or oviposition and nesting sites. Improving our knowledge of the algorithms that animals use
26 during these search efforts represents a critical step towards understanding how organisms function
27 by connecting neuroscience, behavior, ecology, and evolution [1]. On the behavior and ecology fronts,
28 countless field studies have helped shape our understanding of the search behavior exhibited by
29 mammals, birds, and fish in the context of optimal foraging theory and satisficing [2,3]. In laboratory
30 environments designed to discover the neural basis underlying these decisions, many efforts have
31 focused on olfactory search of organisms including mice [4], insects [5], and crustaceans [6] (for a
32 review, see [7]). To move the field forward, there is a growing push to connect laboratory and field

33  experiments. Perhaps surprisingly, the unassuming fruit fly, *Drosophila melanogaster*, has emerged as a

34  prime model for bridging this gap. Evidence is mounting that despite their numerically simple nervous

35  systems, these creatures are capable of forming visual [8,9] and olfactory memories [10–12], and

36  possess an internal representation of their compass heading with respect to visual cues [13–16]. How

37  these adaptations are involved in search, however, remains an area of active research.

38

39  Most natural environments consist of a patchwork of potential resources with a fractal-like

40  distribution, demanding multiple scales of search: long-range, intermediate, local, and nutrient driven.

41  Long-range search for a *Drosophila* consists of flying up to 10 km across the desert to find a new oasis

42  [17,18], initially relying on celestial cues [19,20], as well as vision and wind [21], until it catches an odor

43  plume to follow [22,23]. Within the oasis a fly begins its intermediate search phase: tracking odor

44  plumes [5,24–27] and approaching visual cues [22,28], often relying on the integration of the two to

45  find a fermenting fruit [29,30]. After landing [31], the fly enters its third phase, local search. Now

46  travelling on foot, the fly continues using odors to navigate the patchiness [32–34], as cracks in the

47  skin serve as entry points, whereas mold renders portions too toxic [35]. After tasting some nutrients,

48  the fly enters its final—nutrient driven—search phase, characterized by a so-called "dance" that is

49  largely driven by idiothetic cues [36–38]. Whether or not the fly finds the nutrients it needs, eventually

50  it will decide to take flight and leave, only to start the process all over again. While each of these phases

51  of search has been subject to recent research efforts aimed at understanding both the behavior and

52  neurobiology, little is known about how these individual phases are connected to one another, and

53  how memories from one phase might influence the next.

54

55  In this paper I simulate a patchy environment by placing three ethanol-emitting objects in a wind

56  tunnel. Individually marked fruit flies are allowed to freely explore these objects over the course of an

57  18 hour period. My results indicate that their search behavior on each individual patch is correlated

58  with the time elapsed between patch visits, suggesting that the intermediate and local phases of search

59  are interconnected through a lossy memory-like process.

60

61  **Methods**

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63  To discover the relationship between decisions flies make during local and intermediate search

64  behaviors in patchy landscapes, I placed three food-barren odorous platforms in a wind tunnel (Fig.

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65  1A-B, [5]). Each platform was set up to constantly emit the attractive odorant ethanol from the center

66  of the platform by bubbling 60 mL/min of air through a 50% ethanol/water solution. The acrylic

67  platforms had a perforated area in the center for emitting the odor, and were all identical in size, shape,

68  color, odor type and concentration. The edge was coated in fluon to prevent the flies from crawling

69  out of view.

70

71  To keep track of individual flies across all three platforms, I painted a dot of colored nail polish on

72  their thorax (Fig. 1C). The flies were cold-anesthetized for the painting, and allowed to recover while

73  being deprived of food, but not water, for 8 hours prior to the experiment start. For each experiment,

74  I used six flies. They were placed in the wind tunnel 6 hours prior to their entrained dusk (relative to

75  a 16:8 light:dark cycle), and allowed to move freely throughout the wind tunnel for 18 hours. When

76  the flies landed on a platform, they were tracked by a machine-vision tracking system described

77  previously [5], with one modification. Every 10 seconds, 18 megapixel color dSLR cameras positioned

78  above each patch photographed the flies. All trajectories were hand-corrected for tracking errors to

79  guarantee their completeness, and manually associated with the correct color identity from the dSLR

80  images.

81

82  **Results**

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84  In a stereotypical search-bout, flies would spend a significant amount of time near the odor source in

85  the center of the platform, while also making periodic forays towards the edge, often circling the

86  perimeter of the object (Fig. 1D). To quantify their behavior, I focused on the distance the flies

87  travelled during their search, rather than the time they spent as was been done previously [5] (see

88  Discussion for rationale).

89

90  The first time the flies' encountered an odor emitting platform, they walked a significantly larger

91  distance compared to the average of their subsequent encounters, provided that they engaged with the

92  odor during that first encounter (Fig. 1E-F). These results suggest that flies might maintain some form

93  of memory about their failure to find food in order to minimize the time wasted on future fruitless

94  search endeavors. But does this "memory" fade with time? To further investigate this hypothesis, I

95  analyzed their behavior at a more granular scale by comparing the distance travelled to the time elapsed

96  before (pre-interval), and after (post-interval), visiting additional platforms (Fig. 2A).

97    Most of the flies explored the odorous patches numerous times, often returning to the same patch
98    several times in a row. Intervals between visits ranged from less than a second, to 16 hours. The
99    distance the flies travelled on the platform while searching is, on a log-log scale, correlated with the
100   time elapsed since they last visited a platform (Fig. 2B). Though significant ($p=4\times10^{-5}$), the positive
101   correlation only explains about 10% of the variance ($R^2=0.1$). The unexplained variance, however, is
102   positively correlated with the amount of time until their next patch encounter (post-interval) (Fig. 2C).
103   That is, if flies walk for a smaller distance than predicted based on their pre-interval, then their post-
104   interval is likely small as well, allowing them to make up for the missed opportunities. Relating the
105   distance travelled on the platform to the mean of the pre- and post-intervals resulted in a stronger
106   positive correlation than either alone ($p=2.6\times10^{-8}$; $R^2=0.17$) (Fig. 2D). To ensure that no individual
107   flies played an outsize role in this conclusion, I recalculated the correlation from Fig. 2D in the case
108   where as many as five (32%) random flies were left out of the analysis. The largest p-value for this
109   resampling analysis was $4\times10^{-5}$, indicating that our conclusion is not biased by a small subpopulation.
110
111   There was no correlation between distance travelled and time of day (p=0.38, Supp. Fig. 1A),
112   indicating that neither hunger nor circadian rhythm were important factors. Instead, these factors
113   likely determine whether the flies initiate take off and plume tracking. I also did not find a correlation
114   between pre- and post-interval times (p=0.23, Supp. Fig. B), suggesting there are no consistent
115   behavioral sequences such as rapidly flitting between platforms.
116

117   **Discussion**
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119   My results indicate that search behavior across different search phases—intermediate scale plume
120   tracking and local search on foot—is inter-connected. These observations raise several discussion
121   points: (1) what internal mechanism is responsible for giving rise to the time-interval correlation (e.g.
122   an interval timer, sensory adaptation, or habituation); (2) what mechanism drives flies' decision to
123   leave a patch; and (3) in what ecological contexts is their behavior advantageous.
124

125   *Mechanisms that could give rise to a measure of time-interval*
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127   Although a number of models for neural encoding of interval-timing have been proposed [39], there
128   is little experimental evidence for minute to hour scale interval-timing. In rats, extended time sense is

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129  encoded in the hippocampus [40–42]. However, the accuracy is modulated by drugs, hormones, and

130  context [43,44]. For insects, the ability to measure time-intervals on the scale of seconds to minutes is

131  open for debate. Parasitoid wasps are capable of learning time intervals [45,46]. Honeybees, however,

132  are not [47,48]. Although their cousins, bumblebees, have been shown to learn fixed time intervals

133  [49], the analysis has been called into question [47]. Instead, their behavior suggests that they may have

134  learned a different strategy that approximates interval timing.

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136  The challenges of reliably encoding time in the nervous system suggest that alternate mechanisms are

137  either wholly, or in part, responsible for giving rise to the observed correlation. One possibility is

138  sensory adaptation, however, this is unlikely given that peripheral olfactory receptor level adaptation

139  occurs on much faster time scales (~0.5 second [50]) compared to the observed behavior. Instead,

140  habituation is a more likely explanation. Prior experiments with ethanol induced startle responses

141  indicate that habituated responses are attenuated on the time scales of 15-30 minutes [51].

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143  *Mechanisms driving the decision to leave*

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145  In ecology, the process of search has been dominated by the field of optimal foraging theory, namely,

146  the Marginal Value Theorem [2] [52] [53] and satisficing [3]. Neither model, however, provides an

147  explanation for the underlying neural mechanisms. A number of "rules of thumb" have been proposed

148  [54,55]. One example is the Threshold Giving Up Time (TGUT) strategy [56] [57]), which states that

149  an animal should continue searching for food in a patch for an amount of time proportional to the

150  quality of the patch. However, because maintaining an accurate sense of time elapsed is challenging, a

151  more likely strategy for insects is to leave after some Threshold Giving Up Distance-travelled (TGUD)

152  has been reached, given the behavioral and neural evidence for insects' ability to count steps [37,58].

153  Assuming some variability, a TGUD algorithm should result in a unimodal distribution of distances

154  travelled, but the shape of that distribution could be either normal, or something with a heavier tail

155  such as a lognormal, or levy distribution. An even simpler heuristic for deciding when to leave a patch

156  was proposed in the context of jumping spiders, termed Fixed Probability of Leaving (FPL) [59],

157  where the animal does not need to keep track of time (or distance) and instead leaves with a fixed

158  probability at each time step (or physical step). An FPL strategy would result in an exponential

159  distribution, where the likelihood of leaving after a short distance travelled is very high, falling off

160  exponentially.

161

162     Can flies' decision to leave be modeled by either the TGUD or FPL strategy? A close look at the

163     distribution of distance travelled suggests that although their behavior is not consistent with an

164     exponential distribution it is consistent with both gamma and lognormal distributions, with parameters

165     that vary as a function of the intervals between visits (Fig. 2E-F). At first glance, these results appear

166     to favor the TGUD model, however, a gamma distribution is equivalent to a sum of exponential

167     distributions. Thus, a simple alternative is that the fly has multiple FPL-like processes operating

168     together, which I term Gamma Probability of Leaving (GPL). To distinguish between the TGUD and

169     GPL models will require connecting the behavior with neural activity.

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171     *Ecological context for time-interval correlated search*

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173     What advantages might a correlation between intermediate and local search behaviors confer? I

174     propose two hypotheses. First, the fly might form a memory, associating the odor with a low likelihood

175     of finding food, thereby improving search efficiency. However, these relationships might change with

176     the environment and season, so it would make sense for such a memory to revert to an innate value

177     over time. The second hypothesis is that their behavior optimizes search in non-homogeneous

178     environments, where potential resources are clustered in groups separated by larger distances. In this

179     case, it would make sense to tailor search time relative to the ease with which a new potential food

180     source can be found. Distinguishing between the memory and unpredictable environment hypotheses

181     will require experiments that manipulate the memories formed by providing food rewards or

182     manipulating the release of neuromodulators such as dopamine.

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185 **Figures**

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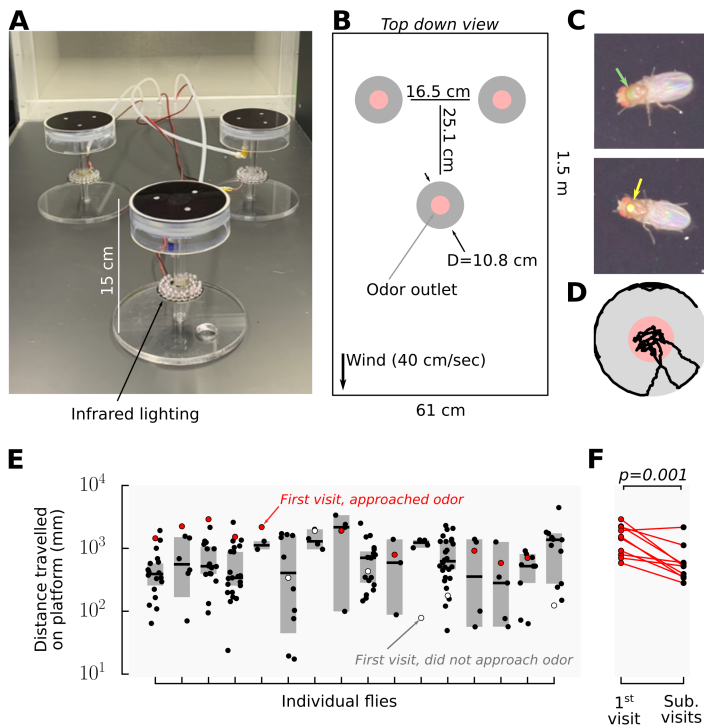


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188 **Fig. 1 | Flies explore odorous objects longer on their first encounter.** (A) Photograph of experimental arrangement
189 of three odor emitting platforms inside a 60x60x120 cm³ wind tunnel. Above each platform was a machine vision camera
190 to track the flies' walking trajectories, and a digital SLR to image their markings. (B) Top down diagram of experimental
191 arrangement, red shading indicates approximate region where ethanol odor was emitted, see [5]. (C) Representative
192 photographs of flies indicating their identifying color spots painted on with nail polish. (D) Representative trajectory on
193 one platform. (E) Distance travelled on the platform by individual flies, shading indicates 95% confidence interval about
194 the mean of all visits after the first one. (F) When flies approached the odor on their first visit, they covered more ground
195 than on average during their subsequent visits (paired T-test; t=4.56; p=0.001).
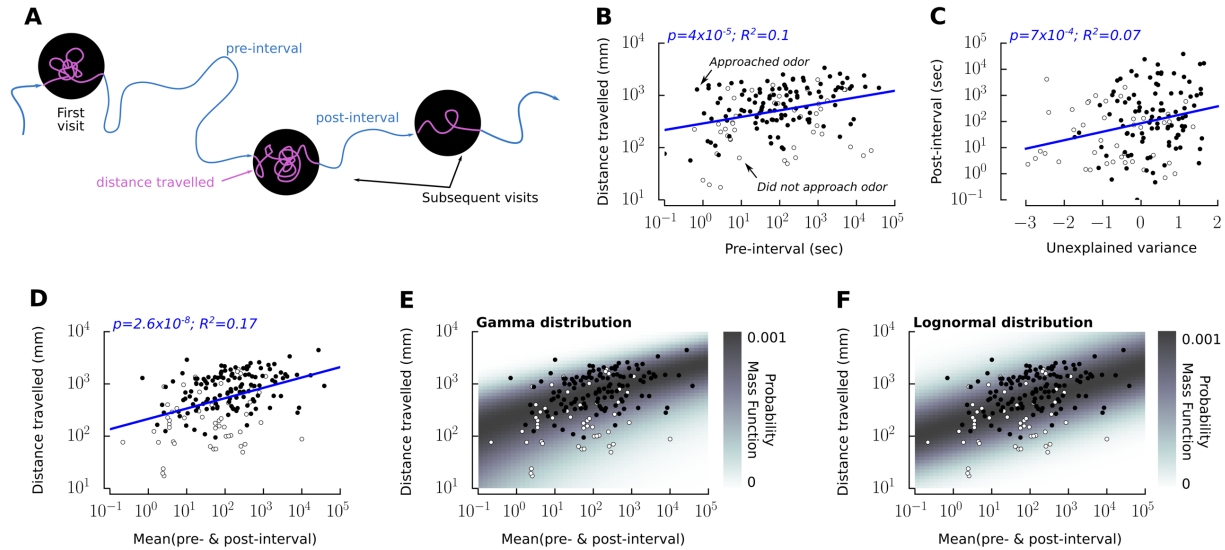
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**Fig. 2 | When flies explore an odorous, but food-barren, object, the distance they travel on the object is proportional to the time elapsed since their last visit to a similar object.** (A) Cartoon of a hypothetical sequence of visits to three odor emitting patches. (B) Distance flies travel on the platform as a function of the time elapsed since their last visit to the same, or different, platform. Black and white colored dots indicate flies that either approached, or did not approach, the odor during that particular visit, respectively. Blue line shows the linear regression, including all data. Excluding flies that did not approach the odor does not change the statistics in any meaningful way. (C) Post-interval time as a function of the difference between the data and linear regression from B. (D) Relating distance travelled to the mean of the pre- and post-intervals improves the strength of the correlation, plotted as in B. The data in D is well modelled by either a gamma (E), or lognormal (F) distribution, with shape and scale parameters that vary with the abscissa.
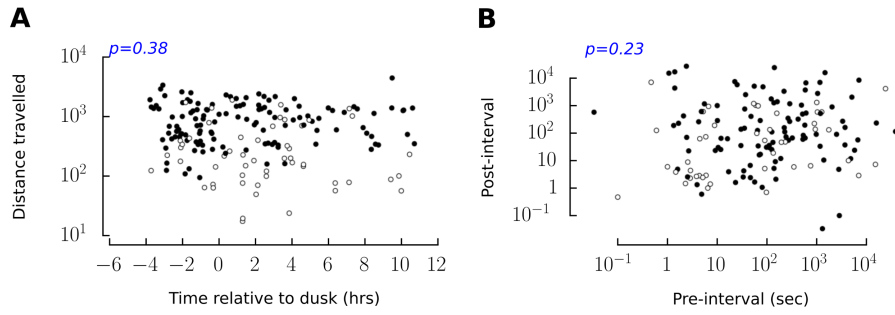
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230 **Supp. Fig. 1** (A) Distance travelled on the platform is not correlated with time of day (p=0.38). (B) Pre- and post-intervals

231 are not correlated with one another (p=0.23). Black and white colored dots indicate flies that either approached, or did

232 not approach the odor during that particular visit.

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245 **References**

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