1 The Foraging-mode Paradigm: A Historical Overview

2 Including a Reevaluation of its Predictions

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6 **Abstract**

7

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# 9 Introduction

10 A central tenet in behavioral ecology is to determine how organisms exploit food in a

11 given environment [(MacArthur and Pianka,](#_bookmark10) [1966).](#_bookmark10) In nature, food is found in patches

12 or lumps that vary in quality or density over time. In response to such environ-

13 mental heterogeneity, organisms adopt certain foraging behaviors that maximize their

14 fitness [(Schoener,](#_bookmark16) [1969).](#_bookmark16) For instance, an active-foraging behavior is attributed to an

15 organism that frequently abandons foraging sites. When the foraging site is rarely

16 abandoned, the organism rather displays a sit-and-wait behavior. This behavioral di-

17 chotomy is currently known as “The Foraging-mode Paradigm”; a categorization that

18 may seem somewhat crude but most researchers would agree that many organisms fall

19 clearly at the extremes of a continuum. A seemingly tireless hummingbird that visits

20 flowers in search of nectar, as opposed to a kingfisher that waits on a perch and swoop

21 in the water when a fish passes by, are perfect examples to illustrate this point.

22 Since the early 1970s, researchers have developed mathematical models to specify

23 which behavior is best suited for an organism to maximize energy intake in a partic-

24 ular environment, leading to the invention of the optimal foraging theory [(Schoener,](#_bookmark17)

25 [1971;](#_bookmark17) [Charnov,](#_bookmark3) [1976).](#_bookmark3) However, the initial application of the optimal foraging the-

26 ory was to explain the evolution of body sizes of organisms with little emphasis on

27 their foraging mode. This theory was later extended in models that explicitly com-

28 pared between foraging modes as alternative strategies among species (e.g., [Vitt and](#_bookmark20)

29 [Congdon,](#_bookmark20) [1978;](#_bookmark20) [Janetos,](#_bookmark8) [1982a).](#_bookmark8) Specifically, the models focused on investigating two

30 main predictions: 1) Organisms should have a simple decision rule for giving up at a

31 foraging site. They should move only when the expected gain from moving surpasses

32 the expected gain from remaining at the foraging site. 2) If individual variation in

33 foraging behavior affects the energetic benefits/costs, differences in growth rate, body

34 size, and reproductive output are expected among individuals. This expectation is

35 supported by the idea that different behavioral strategies determine the life histories

36 of organisms by limiting their acquisition and allocation of energy to vital processes.

37 An allocation tradeoff suggests that an increment in energy allocated to one function

38 results in a decrement in energy allocated to other functions. Thus, an individual that

39 acquire greater surplus energy may growth faster, have both a smaller body size and

40 greater reproductive output [(Stearns et al.,](#_bookmark19) [1992;](#_bookmark19) [Roff,](#_bookmark15) [2002).](#_bookmark15)

41 Such predictions were instantly evaluated in a few elegant works fueled by the

42 increasing interest in behavioral ecology at the time. The first empirical evidence

43 derived from field studies of lizards [(Vitt and Congdon,](#_bookmark20) [1978;](#_bookmark20) [Vitt and Price,](#_bookmark21) [1982).](#_bookmark21)

44 In a comparative analysis of reproductive output among species, the authors showed

45 that active foragers had lower reproductive investment than did sit-and-wait foragers,

46 with the rationale being that carrying a voluminous clutch while pursuing a prey in-

47 creases the probability of being killed by a predator or reduces the foraging efficiency.

48 Interestingly, a different study on orbweaver and sheetweb weaver spiders indicated

49 that active foragers are subject to lower energetic costs [(Janetos,](#_bookmark9) [1982b).](#_bookmark9) Orbweavers

50 seemed to evaluate whether to stay or leave the web based on the abundance of prey

51 they capture in a day (i.e., the quality of the foraging site). As expected from active

52 foragers, orbweavers leave the web in search of a better foraging site when the availabil-

53 ity of prey is low. By contrast, sheetweb weavers seemed to be sit-and-wait predators,

54 staying on the web for a longer time and only leaving it at random. Surprisingly, the

55 author showed that sheetweb weavers pay a much higher energetic cost for construct-

56 ing a new web from body reserves than do orbweavers. It is important to point out

57 that the analyses described above might have been confounded by the phylogenetic

58 relationships among species. However, interspecific studies at the time unavoidably

59 suffered from such bias as the development of phylogenetic comparative methods was

60 only available until 1985 with the foundational paper published by [Felsenstein](#_bookmark7) [(1985).](#_bookmark7)

61 In parallel to the findings described above, some works on the behavioral polymor-

62 phism in freely foraging *Drosophila melanogaster* led to the discovery of the foraging

63 gene (“*for* ”, [Sokolowski,](#_bookmark18) [1980).](#_bookmark18) This pioneering study showed that individual larvae

64 differed in how far they traveled while foraging. Accordingly, individuals could be clas-

65 sified into rover or sitter behavioral morphs, with rovers traveling significantly longer

66 distances than sitters while on a feeding substrate. The discovery of the “*for* ” gene was

67 particularly important because it paved the way for researchers to better understand

68 the genetic basis of foraging behavior. For instance, early genetic analyses mapped

69 the difference in foraging behavior to chromosome 2, with rover showing genetic dom-

70 inance over sitter [(de Belle and Sokolowski](#_bookmark6), [1987).](#_bookmark6) Later work also localized the “*for* ”

71 gene to the cytological location 24A2–24A4 on the left arm of chromosome 2 [(de Belle](#_bookmark5)

72 [et al.,](#_bookmark5) [1989).](#_bookmark5) The prevailing ecological perspective of foraging behavior at the time

73 was then complemented with the increasing interest in the study of the genetic basis

74 of such behavioral polymorphism.

75 As more data became available, novel insights into the evolution of foraging behav-

76 ior flourished in recent decades. With the advent of next-generation sequencing, for

77 example, we now know that geographic and ecological factors are responsible for impor-

78 tant genetic differences at “*for* ” across populations of *D. melanogaster* (P[adilla Perez,](#_bookmark12)

79 [2024).](#_bookmark12) We also know that an epigenetic regulator of the “*for* ” gene (the G9a methyl-

80 transferase) is responsible for rover–sitter differences in adult foraging behavior [(An-](#_bookmark0)

81 [reiter et al.,](#_bookmark0) [2017](#_bookmark0)). Evidence from behavioral ecology studies have shown conflicting

82 results though. While early studies suggest that active foragers have lower repro-

83 ductive investment than do sit-and-wait foragers [(Vitt and Congdon,](#_bookmark20) [1978;](#_bookmark20) [Vitt and](#_bookmark21)

84 [Price,](#_bookmark21) [1982),](#_bookmark21) more recent evidence show either no differences in the life history be-

85 tween the two behavioral strategies [(Mesquita et al.,](#_bookmark11) [2016),](#_bookmark11) or an opposite pattern to

86 what most researchers have previously found. That is, there is an interaction between

87 reproductive effort and body size such that active foragers have greater reproductive

88 output than do sit-and-wait foragers at large body sizes (P[adilla Perez et al.,](#_bookmark13) [2022).](#_bookmark13)

89 Therefore, the expectation of differences in the life history between active foragers and

90 sit-and-wait foragers requires further investigation.

91 Importantly, the vast majority of the evidence described above come from interspe-

92 cific studies, which enable one to make plausible inferences that can be evaluated at the

93 intraspecific level. Given its well-known behavioral polymorphism, *D. melanogaster*

94 provides a good opportunity to further investigate long-standing predictions of the

95 foraging-mode paradigm. Here, we aimed to evaluate two predictions: 1) The dis-

96 tance travel by active foragers (rovers) and sit-and-wait foragers (sitters) may be de-

97 termined by the distribution of food in the environment. 2) If variation in foraging

98 behavior affects the energetic benefits/costs, differences in the life history (e.g., growth

99 rate) should be observed between the two behavioral strategies. Our results suggest

100 that environmental heterogeneity alters the foraging behavior of individuals sufficiently

101 enough to stimulate a faster growth when food is lumpy in the environment. However,

102 growth rate seems to be the same between active foragers and sit-and-wait foragers

103 regardless of the distribution of food in the environment.

# 104 Materials and Methods

105 ***Fly strains***

106 The rover (*forr*) and sitter (*fors*) strains used in the experiments have isogenized *forr*

107 or *fors* 2nd chromosomes, sharing isogenized X and 3rd chromosomes from the rover

108 B15 strain as described in [Bauer and Sokolowski](#_bookmark2) [(1985)](#_bookmark2) and [Sokolowski](#_bookmark18) (1980). We

109 maintained the flies at 25°C, in a 12:12h light/dark cycle at 60% relative humidity with

110 lights on at 08:00h. We reared populations of flies in 8oz round-bottom Drosophila

111 bottles, with a standard yeast-sugar-agar medium as suggested by [Anreiter et al.](#_bookmark1)

112 [(2016).](#_bookmark1) Before the beginning of the experiments, we transferred the flies into holding

113 empty bottles and capped them with grape plates containing a small amount of dry-

114 active yeast to stimulate reproduction. We removed the grape plates from the bottles

115 22 hr after they were set up and discarded all larvae from the seeded grape plates using

116 a dissecting probe. We then incubated the eggs that remained in the grape plates for

117 4 hr in standard conditions as described earlier. After 4 hr, we picked L1 larvae per

118 strain from the grape plates and placed them on food plates (i.e., yeast-sugar-agar

119 medium). Lastly, we collected the testing larvae (L3) about 10 hr before wandering,

120 which generally corresponds to 72-96 hr after hatching [(Anreiter et al.,](#_bookmark1) [2016).](#_bookmark1)

121 ***Experimental design for prediction 1: Distance traveled by active foragers***

122 ***and sit-and-wait foragers***

123 We measured the distance traveled by L3 larvae while foraging in two types of envi-

124 ronments that differed in the distribution of food. While one environment consisted of

125 yeast paste distributed in patches on Drosophila agar medium, the other one contained

126 only a lump of the paste on the medium. We set up these environments in 32 *×* 10 mm

127 petri dishes. To make the patchy environment, we used a 12*ml* insulin syringe to pour

128 small drops of dry-active yeast mixed with water at a 1:2 ratio (weight to volume).

129 We followed the same procedure to make the lumpy environment, but this time we

130 poured the paste in such a way that a lump formed at the center of the plates (see

131 supporting material for details). Importantly, the consistency of the paste, the volume

132 used (2*µl*), and the configuration of the food were the same among the test plates (see

133 supporting material for details).

134 We collected individual L3 larvae and placed them in the test plates for 1 hr period

135 necessary for acclimatization. To do this, we first randomized the strain, the type

136 of environment, and the position on the plates where the larvae were released. To

137 randomize these factors, we used the “*sample*” function available in the free software

138 R v.4.3.2 (2023-10-31, [R Core Team,](#_bookmark14) [202](#_bookmark14)3), which enabled us to pick a sample of a

139 specified size (*n* = 1 in this case) from a vector of predefined elements (e.g., a vector of

140 two characters: “rover” and “sitter”). To run the experiments, we placed the plates in

141 an incubator set up at 25°C and 60% relative humidity. We then recorded the larvae

142 for 30 min, using a camera held 30 cm above the plates. In each trial, we recorded four

143 plates simultaneously as indicated by Figure S**??**. The experiments yielded a sample

144 size of 106 larvae; 58 of which were tested in a patchy environment (*n* = 27 rover,

145 *n* = 31 sitter), and 51 tested in a lumpy environment (*n* = 29 rover, *n* = 22 sitter).

146 After the end of the experiments, all of the larvae were transferred back to food plates

147 where they continued to develop.

148 ***Experimental design for prediction 2: Growth rate of active foragers and***

149 ***sit-and-wait foragers***

150 To measure growth rate, we let L3 larvae develop in two different environments with

151 food distributed in patches and lumps, as described earlier. We measured growth rate

152 as the difference between the initial mass and the final mass of the larvae over a 24

153 hr period. To record the initial mass, we gently washed the larvae with 1-2 ml of

154 water and dabbed them dry with a paper towel to avoid any confounding factor when

155 weighing the larvae. We then weighed the larvae using a micro-analytical balance

156 (Metler Toledo Model XPR6UD5), and transferred them into the test plates. The test

157 plates were placed in an incubator set up at 25°C and 60% relative humidity. After 24

158 hr, we weighed and recorded the final mass of the larvae following the same procedure

159 described above.

160 ***Data analysis***

161 The experiments performed to evaluate the predictions of this study corresponded to

162 a 22 factorial design. This particular design is referred to as a 22 (read “two-by-two”)

163 factorial design because it combines two independent variables, each of which has two

164 levels. For instance, fly strain can be viewed as a factor with two levels: “rover”

165 and “sitter”. Likewise, the type of environment can also be encoded as a factor with

166 two levels: “patchy” and “lumpy”. Factorial designs are a simple, yet elegant, way of

167 comparing the main effects of multiple independent variables and exploring possible

168 interaction effects.

169 Based on the notation used in this full factorial design, the two-way ANOVA model

170 represents the most appropriate model to analyze the data. Accordingly, we fitted two

171 ANOVA models as follows: 1) The first model described the effects of the strain

172 and the environment on the distance traveled by the larvae. As mentioned earlier,

173 we used a camera to record the movement patterns of the larvae. To analyze the

174 recordings, we used the free software AnimalTA v.2.2.1 [(Chiara and Kim,](#_bookmark4) [2023);](#_bookmark4) a

175 video-tracking software that enabled us to analyze numerous videos recorded under

176 the same conditions. 2) The second model described the effects of the strain and the

177 type of environment on the growth rate of the larvae. In both models we not only

178 tested the main effects, but also the interactions between the independent variables.

179 To do so, we used the function “*lm*” available in the free software R v.4.3.2 (2023-

180 10-31, [R Core Team,](#_bookmark14) [2023).](#_bookmark14) To evaluate the models’ goodness of fit, we considered

181 the adjusted *R*2 of the models, which indicates the percentage of variance that the

182 models could explain given the data. To produce a good visualization of our results

183 and ensure that they are fully reproducible, we carried out all the analyses in the free

184 software for statistical computing R v.4.3.2 (2023-10-31, [R Core Team,](#_bookmark14) [2023](#_bookmark14)).

185 **Results**

186 **Discussion**

187 **Acknowledgements**

# 188 Data Accessibility Statement

189 A fully reproducible workflow of the data analyses, including R scripts and additional

190 supporting material, is available in the following repositories: Github .

# 191 Conflict of interest

192 The authors have declared no competing interests.

# 193 Author Contributions

194 Dylan Padilla: Conceptualization, data curation, and formal analysis. Writing – orig-

195 inal draft, writing – review and editing. The authors agreed to be held accountable

196 for the work performed herein.

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250 **Figures with captions**