1   # How predator hunting-modes affect prey behaviour: Capture

2   # deterrence in *Drosophila melanogaster*

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16   Links to videos (also available in supplement)

17   Abdominal Lifting http://dx.doi.org/10.6084/m9.figshare.1185638

18   Stopping Behaviour http://dx.doi.org/10.6084/m9.figshare.1185639

19   Retreat http://dx.doi.org/10.6084/m9.figshare.1185640

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21 **ABSTRACT**

22 Hunting mode or the distinct set of behavioural strategies that a predator employs while

23 hunting can be an important determinant of the prey organism's behavioural response.

24 However, few studies have considered the predator's hunting mode while describing

25 differences in anti-predatory behaviours of a prey species. Here we document the

26 influence of active hunters (zebra jumping spiders, *Salticus scenicus*) and ambush

27 predators (Chinese praying mantids, *Tenodera aridifolia sinensis*) on the capture

28 deterrence anti-predatory behavioural repertoire of the model organism, *Drosophila*

29 *melanogaster*. We hypothesized that *D. melanogaster* would reduce overall locomotory

30 activity in the presence of ambush predators, and increase them with active hunters. First

31 we observed and described the behavioural repertoire of *D. melanogaster* in the presence

32 of the predators. We documented three previously undescribed behaviours- abdominal

33 lifting, stopping and retreat- which were performed at higher frequency by *D.*

34 *melanogaster* in the presence of predators, and may aid in capture deterrence. Consistent

35 with our predictions, we observed an increase in the overall activity of *D. melanogaster*

36 in the presence of jumping spiders (active hunter). However, counter to our prediction,

37 mantids (ambush hunter) had only a modest influence on activity. Given these new

38 insights into *Drosophila* behaviour, and with the genetic tools available, dissecting the

39 molecular mechanisms of anti-predator behaviours may now be feasible in this system.

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41

1   **INTRODUCTION**
2
3   Predation, a ubiquitous selective force, gives rise to and determines the nature of

4   defensive traits in prey populations[1-7]. Predator hunting-modes, i.e., the set of

5   behaviours that predators employ to pursue and capture their prey [8-10], have been

6   shown to induce distinct prey responses [10,11] that in turn influence the productivity of

7   ecological communities. For example it has been found that habitats dominated by active

8   hunters had lower species evenness and higher above-ground net primary productivity

9   compared to habitats dominated by ambush hunters [10]. The authors suggest the

10   observed differences in prey productivity to be driven by hunting mode specific trade-

11   offs between foraging and seeking refuge [10]. However, studies describing the effects of

12   predators on prey traits [12-14] have often ignored the role of predator hunting-mode.

13   In this study we investigate segregating differences in the anti-predatory

14   behavioural repertoire of the model fruit fly, *Drosophila melanogaster*, in response to

15   two predator species differing in hunting modes. Based on [11], we predicted that fruit

16   flies, in the presence of a familiar predator, would exhibit hunting-mode specific

17   modifications in activity levels. We used *D. melanogaster* because, although it is one of

18   the most well-studied model organisms, there is a relative paucity of information

19   regarding *D. melanogaster's* natural ecology and behaviour, including habitat, food

20   resources, and natural enemies (but see [18-24]). Meanwhile, though anti-predator

21   behaviours in general are targets of selection in prey [2,15-17], the genetic bases of such

22   behaviours have seldom been investigated due to lack of molecular genetic tools. Given

23   the range of genetic and genomic tools available for *D. melanogaster,* along with its

24   complex behavioural repertoire and suitability for experimental evolution, understanding

25   the anti-predatory behaviours persisting in a natural population of the fruit fly brings us

26   one step closer to deciphering the molecular mechanisms of evolutionary response to

27   predation as a selective agent.

28   Previous work has examined the effects of natural enemies on population and

29   community structures of *Drosophila spp*. For example, Worthen et al [25] studied the

30   effects of predation by staphylinid coleopterans on the coexistence of three *Drosophila*

31   species, and Escalante et al [26] showed that ant predators regulate population densities

32   of wild *D. starmeri* (cactophillic fruit fly). Additionally, in *D. melanogaster per se*, the

33   role of parasites in influencing larval as well as adult behaviour has been extensively

34   studied [17,27-29]. Despite this literature, we know little about the predators of *D.*

35   *melanogaster* adults in the wild, nor the nature of anti-predatory behaviours segregating

36   in natural populations.

37   We documented the influence of two predators, the zebra jumping spider (*Salticus*

38   *scenicus*) and juvenile Chinese praying mantids (*Tenodera aridifolia sinensis*) on the

39   capture-deterrence behaviours of *D. melanogaster* individuals derived from a wild-caught

40   population. The zebra spider is an active hunter; locating prey visually (with an extensive

41   visual field attained by antero-medially positioned simple eyes) [30,31]. Where as

42   mantids are generally ambush predators; waiting for prey to enter their attack range [32].

43   Despite numerous differences, Zebra spiders and juvenile Chinese mantids are similar in

44   two relevant ways. First, both species primarily detect prey visually [33-36] and are

45   likely incapable of depth perception when their prey item is motionless [36,37]. Second,

46   small adult diptera account form a substantial proportion of the diet of both predators in

47   the wild [38,39].

48   Based on the findings of Schmitz [11], we predicted that fruit flies, in the

49 presence of a familiar predator, would exhibit hunting-mode specific modifications in

50 activity levels. To maximize distance from the actively hunting spider, our prediction was

51 that flies would increase their overall activity levels, whereas, to reduce the probability of

52 encountering a stationary threat (the mantid, an ambush predator), we expected flies to

53 decrease overall activity.

54   Under controlled laboratory conditions, we documented the behaviours of

55 individual adult *D. melanogaster* with and without the two predator species. Our results

56 suggest that in the presence of zebra spiders, *D. melanogaster* increases its overall

57 locomotory activity, performs a distinct "stopping" behaviour and substantially increases

58 the performance of a newly described abdominal lifting behaviour (the function of which

59 is as of yet unknown). Counter to our prediction though, *D. melanogaste*r's locomotion,

60 and most other behaviours are not substantially altered in the presence of mantids.

61 However, upon direct encounter with a mantid, many individuals of *D. melanogaster*

62 perform (a previously undescribed) retreat behaviour- a response not generally elicited by

63 jumping spiders. We discuss our results within the broad context of conditionally

64 expressed behaviours as they relate to predator hunting mode, as well as specifically with

65 respect to broadening our understanding of the behavioural ecology of *D. melanogaster*.

66

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67
68   **METHODS**

69   ***Drosophila Population and Culture Conditions***

70   The *Drosophila melanogaster* population used in this study originated from a

71   natural population at Fenn Valley Vineyards in Fennville, Michigan (GPS coordinates:

72   42.57, -86.14) during the summer of 2010. A lab population (henceforth referred to as

73   FVW) was initiated from this collection using the progeny of over 500 single-pair

74   matings of field caught *D. melanogaster* as well as wild caught males. This design

75   allowed us to screen out the sympatric congener, *D. simulans,* which was present in our

76   collections at a frequency of about 5%. Screening involved setting up single pair mating

77   in vials and discarding all lines with *D. simulans*-like genital morphology. After

78   screening, ~1500 individuals were placed into cage (32.5cm^3, BugDorm BD43030F) to

79   establish the FVW population. The population is currently maintained in this cage at an

80   adult density ~ 3000 individuals in a room maintained at 23^0C (+/- 1^0C), and 40-70% RH.

81   Adults were allowed to lay eggs in 10 bottles with 50-60 ml of a standard yeast-cornmeal

82   food for 2-3 days. These bottles were then removed and kept in a Percival incubator

83   (Model: I41VLC8) at 24^0C and 65% RH throughout the larval stages. All flies and larvae

84   were maintained in a 12 hr light/dark cycle with lights on at 08:00 hours.

85   For the experiments, pupae were collected 24 hours before they emerged as

86   adults. Pupae were removed from bottles using forceps and individual pupae were placed

87   into 1.5 ml microcentrifuge tubes. Each tube was pre-filled with ~ 0.5 ml of yeast-

88   cornmeal food and its cap was punctured for gas exchange. Upon emergence, adult flies

89   were sexed visually without anesthesia and housed in these tubes in the incubator until

90   needed for behavioural assays. Age of flies used in behaviour analysis was 3-7 days. By

91   using socially naïve flies in our assays, we were able to establish a consistent baseline of

92   social experience among all individuals, allowing us to eliminate the potentially

93   confounding influence of variation in social experience on behaviour that is well-

94   documented in *Drosophila* [40-44].

95

96   ***Spiders***

97   *S. scenicus* individuals were collected throughout the spring/summer of 2012 on

98   the campus of Michigan State University. Spiders were housed individually in vials in a

99   room maintained at 23^0C (+/- 1^0C) and 30-50% RH and fed ~5 *D. melanogaster* a week.

100   Prior to use in behavioural assays, spiders were starved for at least 48 hours. Each spider

101   was used in only a single behavioural assay.

102

103   ***Mantids***

104   Mantid egg cases were both collected near the campus of Michigan State

105   University as well as ordered from Nature's Control (Medford, Oregon). Mantid egg

106   cases were stored at 4^0C and transferred to 25^0C and 70% RH for hatching. Given the

107   substantial changes in mantid body size across moults [45], only first instar nymphs were

108   used for experiments. Prior to behavioural assay, mantids were starved for at least 24

109   hours and each mantid was used only once.

110

111   ***Behavioural Assays***

112   All assays were performed 1-4 hours after the incubator lights came on in the

113   morning (08:00). Behavioural assays were recorded with an Aiptek AHD H23 digital

114   camcorder attached to a tripod under a combination of natural and fluorescent light that is

115   present in the room wherein the FVW population and spiders are maintained. For each

116   predator (spiders and mantids), we recorded the behaviour for each of 15 male and 15

117   female socially naïve, virgin flies (collected as described above). We used a chamber

118   constructed from the bottom of a 100 x15mm petri dish inverted on top of a glass plate

119   with a sheet of white paper beneath to maximize the visibility of flies and predators.

120   For each assay, an individual fly was aspirated into the chamber and allowed to

121   acclimate for 5 minutes. After this acclimation period, flies were recorded for 5 minutes.

122   A single spider or mantid was then introduced to the chamber and behaviours were

123   recorded for an additional 10 minutes or until capture. The chamber was washed with 10-

124   30% ethanol and rinsed with reverse osmosis water after each assay to remove olfactory

125   cues.

126   .

127   ***Behaviours Recorded***

128   All *Drosophila* behaviours were categorized and analysed as either "states" or

129   "events". Behavioural states have measurable duration and are mutually exclusive with

130   other states (e.g. individuals cannot simultaneously walk and run). Behavioural events

131   are discrete behaviours that occur instantaneously and are also mutually exclusive with

132   each other (e.g. turning versus jumping) but not always mutually exclusive with

133   behavioural states. For example, an individual could perform a wing display (event) while

134   simultaneously walking (state), but it could not jump (event) while simultaneously

135   running (state). In this study we treated flying as an event because the structure of the

136   experimental chamber was prohibitive to flight. Attempted flight by *D. melanogaster*

137   often resulted in landing due to collision with a wall of the petri dish. We also recorded

138   when a fly was not visible (occluded) to the observers analysing video. We recorded a

139   total of 6 discrete events and 5 behavioural states in *D. melanogaster* in response to

140   predation by spiders and mantids (Table 1). In order to interpret an individual fly's

141   behaviour in the context of predatory encounters, we designated two keys to describe the

142   location of the predator in regard to its interactions with the fly. As flies might alter their

143   behaviour when a predator is within striking distance, we recorded predator location

144   based on whether or not it was within striking distance of the fly (~ 5mm from the

145   spider/mantid, also see *Spider location/ Mantid location* in Figure 1).

146

147   ***Video Processing***

148   Recorded behaviours were viewed with VLC media player (version 2.0.3) and

149   analysed by two observers using a manual event recorder, JWatcher V1.0 software [46].

150   One observer (A.P.) viewed each video and verbally announced the occurrence of

151   behaviours while the other observer (C.P./ M.C.) recorded the occurrence of these

152   behaviours with JWatcher. Because *Drosophila* anti-predatory behaviours are often

153   complex and occur rapidly, we analysed all videos at 0.5X speed.

154

155   ***Controlling for effects of season and disturbance***

156   We conducted all spider observations between October and December 2012 and all the

157   mantid observation between March and May 2013. To confirm that predator species-

158   specific behavioural differences were not confounded with seasonal differences in

159   behaviour, we performed 6 additional assays (alternating between spider and mantid

160   treatments) within the span of one week. Following a spider assay, the plates were wiped

161   down with 30% ethanol followed by a rinse with RO water before a mantid assay was

162   conducted.

163   Additionally, the process of adding a predator to the arena invariably resulted in a

164   disturbance that likely startled the fly (unrelated to the presence of a predator). To

165   confirm that behaviours induced by this disturbance were not confounded with predator

166   induced behavioural differences, we performed 3 control assays. Here, after 5 minutes of

167   acclimatization without a predator (see above for more details), the arena containing the

168   fruit fly was disturbed gently (~ magnitude of disturbance caused by the addition of a

169   predator). For all controls, video processing and behaviours recorded were identical to

170   mantid and spider treatments described above. See Supplement b, S1 for a detailed

171   description of these control experiments and their results.

172

173   ***Data processing and statistical analysis***

174   A custom Python script was used to parse Jwatcher formatted data files into a

175   comma-separated-value (CSV) file for analysis in **R** (version 3.0.1).

176   To analyse the effects of predator state (i.e., presence or absence of predators) on

177   the time dedicated to locomotory behavioural states, and number of occurrence for

178   behavioural events, we fit mixed effects models (using both *glmer* function; lme4

179   package version 1.0-5, and *MCMCglmm* function; MCMCglmm package version 2.17)

180   with predator state, total duration of assay with and without a predator (duration), sex,

181   temperature and recording time as fixed effects, and individual by predator state and date

182   as random effects . Formally, the model was:

183

184
$$y \sim \beta_{0ij} + \beta_1 PS + \beta_2 D + \beta_3 Ag + \beta_4 T + \beta_5 ST + \beta_6 Sx + \varepsilon$$

185

186 Where **y** is a vector of time spent in a behavioural state. β_1 is the regression coefficient for

187 predator state, β_2 is for duration in each predator state, β_3 is for age of the fly, β_4 is for

188 temperature, β_5 is for time at which assay was started, β_6 is for sex of the fly and β_7 is for

189 date on which the assay was performed. We estimated random effects for individuals

190 including variation in response to predator state and duration of assay, and we fit an

191 independent random effect for date. Thus we fit a repeated effects (longitudinal) mixed

192 effects model allowing for variation among individuals for the influence of predator

193 presence and duration of assay where for the ith individual

194
$$\begin{pmatrix} \beta_{0i} \\ \beta_{1i} \\ \beta_{2i} \end{pmatrix} \sim MVN \left(\begin{bmatrix} \mu_{\beta_0} \\ \mu_{\beta_1} \\ \mu_{\beta_2} \end{bmatrix}, \begin{bmatrix} \sigma^2_{\beta_0} & \sigma_{\beta_0,\beta_1} & \sigma_{\beta_0,\beta_2} \\ \sigma_{\beta_0,\beta_1} & \sigma^2_{\beta_1} & \sigma_{\beta_1,\beta_2} \\ \sigma_{\beta_0,\beta_2} & \sigma_{\beta_1,\beta_2} & \sigma^2_{\beta_2} \end{bmatrix} \right)$$

195

196 and (independent of the above)

197 $\beta_0 \sim N(0, \sigma^2_j)$ where j = 1 … date

198

199 Preliminary analyses were inconsistent with the need to fit higher order interactions

200 among fixed effects, so interaction terms were not considered further.

201 To test for non-random associations in the temporal structure of behavioural

202 patterns we constructed transition frequencies using the "msm" library (version 1.2) [47]

203 in **R**. To test for both for first order Markov processes between behaviours (transition

204 probabilities), as well as the influence of predator presence on these transition

205   probabilities, we fit log-linear models with the transition frequency matrices [48] using

206   glm in **R**. As advocated by [48,49] we fit a saturated log-linear model (with lag0, lag1

207   and PredState as the effects in the model) and tested the influence of deleting the terms

208   (i.e. third order interaction of main terms) on change in deviance. We used modified "Z-

209   scores", adjusted using sequential Bonferroni to assess the deviation of particular cells in

210   the transition frequency matrix from expected values (assuming independence). For the

211   visual transition probability matrices, we combined the behavioural event "pause" with

212   the behavioural state "stop" because 1) we wanted to reduce the complexity of the matrix

213   and 2) the main difference between the two behaviours is that pause is instantaneous and

214   stop has duration. All transition diagrams were constructed in Inkscape [50](version

215   0.48.2).

216

217

Table 1 Names and descriptions of all observed behaviours. Videos are provided at the end of Supplement b.

| Behaviour	Description
Abdominal lift (ab)	Momentary rearing up on abdomen (see video 1)
Fly	Moving through space by wing use
Jump	Instantaneous movement between points without wing use
Pause	Noticeable period of inactivity; transitional
Turn	180 degree change in orientation without change in position
Wing display (wd)	Momentary lifting up of wings without singing or vibration
Groom	Running legs over any body part-often while otherwise stationary
Walk	Movement through space by ambulation
Run	Rapid movement through space by ambulation
Stop	Immobile (see video 2)
Retreat	Walking in reverse upon encounter with an object (like a predator) (see video 3)

217
218 **RESULTS**

219   From pilot observations (not included in analysis), we (I.D., A.P. and C.P.)

220 catalogued and described *Drosophila melanogaster* behaviours observed in the presence

221 of a predator (Table 1). Among the behaviours listed in Table 1, abdominal lifting (ab,

222 supplement b, video 1) and retreat (supplement b, video 3), to our knowledge, have not

223 been previously described in *D. melanogaster* literature.

224

225 ***Flies perform a range of anti-predatory behaviours in response to a zebra spider***

226   To visualize each individual fruit fly's response to the presence of a zebra

227 jumping spider, we generated ethograms (see Figure 1a and Supplement a). For the two

228 predator states (spider present and spider absent) we measured the mean proportion of

229 time dedicated to each behavioural state, as well as the number of occurrences per minute

230 for each behavioural event. When a spider was present, *D. melanogaster* increased the

231 proportion of time it spent walking and running while grooming less (Figure 2 and

232 Supplement b Figure S1). While they were observed at low frequencies prior to the

233 addition of spiders, *D. melanogaster* substantially increased the frequency of pauses,

234 abdominal lifts, jumps and flights (per minute) in the presence of spiders (Figure 2,

235 Supplement b Figure S3). Time allocated to "stopping"; a motionless state that likely aids

236 in capture deterrence (see Videos 2, Supplement c) also increased significantly in the

237 presence of spiders (Supplement b Figure S2). However, when interacting with spiders,

238 flies were only observed to perform the "retreat" behaviour once (of 30 individuals).

239   Given the design of our experiment, we were able to model the degree to which

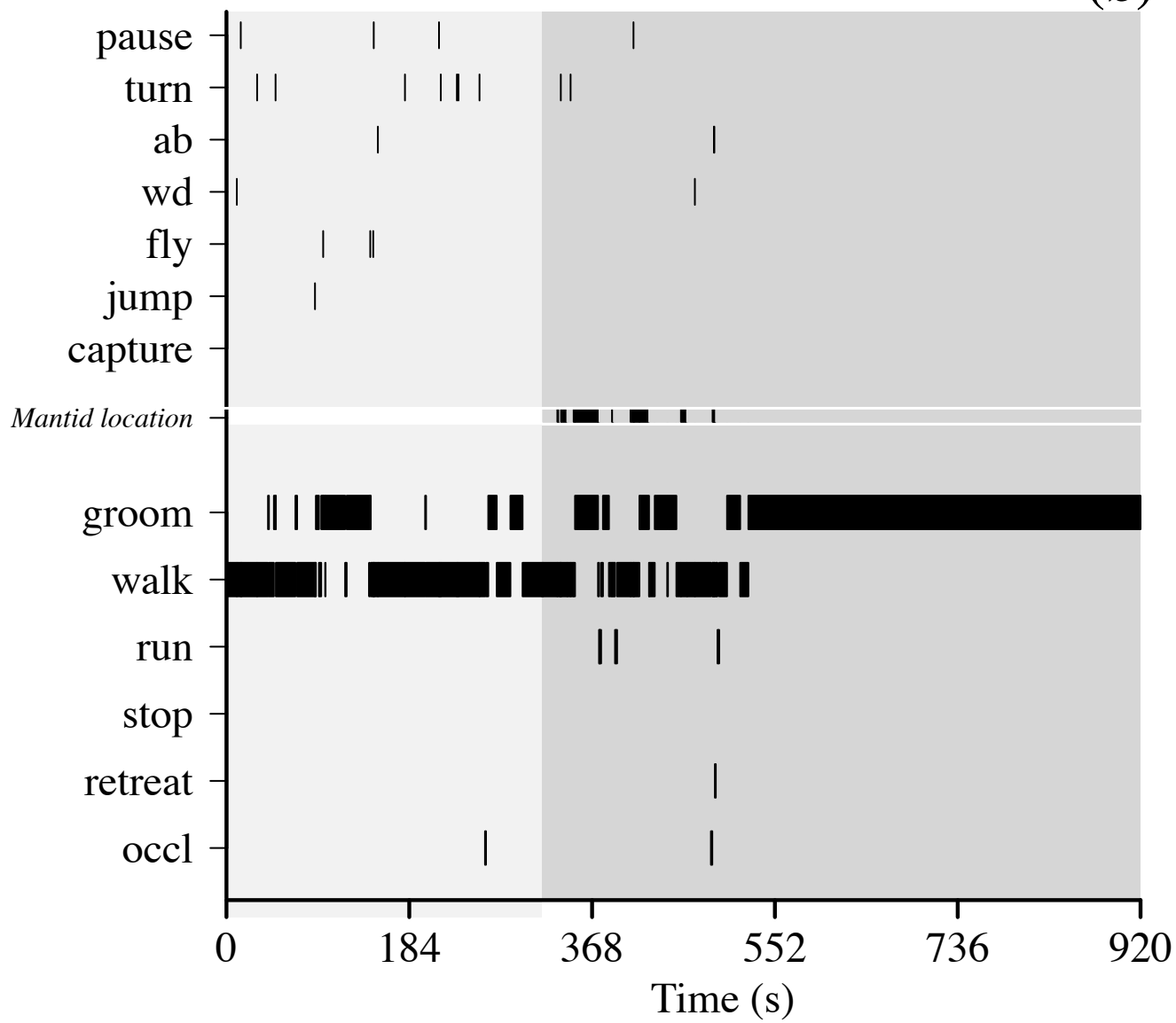240 individuals varied in their responses to the jumping spiders. Individuals varied greatly

(a)
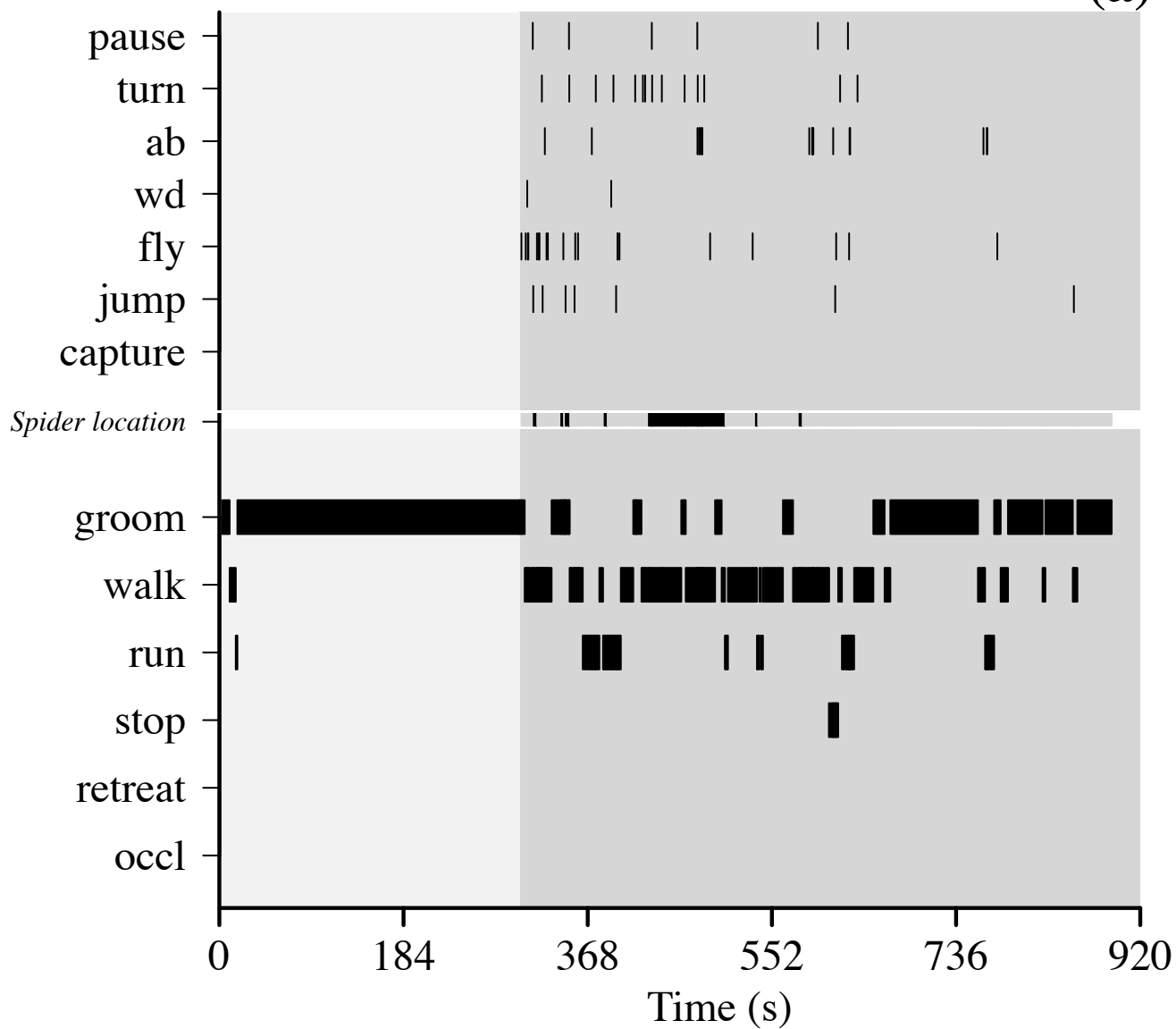
(b)

Figure 1

D. melanogaster used a greater proportion of its behavioural repertoire and performed each behaviour at a higher frequency in the presence of a jumping spider than in the presence of a juvenile mantid. ***a)*** Representative ethogram of a male, 4 day old *D. melanogaster* in response to a zebra jumping spider. ***b)*** Ethogram of a male, 5 day old *D. melanogaster* in response to a juvenile Chinese paying mantid. Light grey background represents time in the arena before the addition of a predator and dark grey background is when the predator was present in the chamber. Each black bar represents the occurrence of a behaviour during the observation period. Top half of the figure (separated by *Predator location*) consists of events and the bottom half consists of states. Because states have duration, the width of each black bar corresponds to the duration of a state. *Predator location* (i.e., *Spider location* in **a** and *Mantid location* in **b**) indicates whether or not the predator was within striking distance of the fruit fly at that time point. This information is relevant only after the predator was added to the chamber (~ 300 s into the assay). Dark grey bars in *Predator location* indicate that the spider was within striking distance and light grey regions indicate that the spider was out of striking distance. *Predator location* is white when the predator is absent from the arena or after successful capture. If capture did not occur, *predator location* remains light grey in colour.
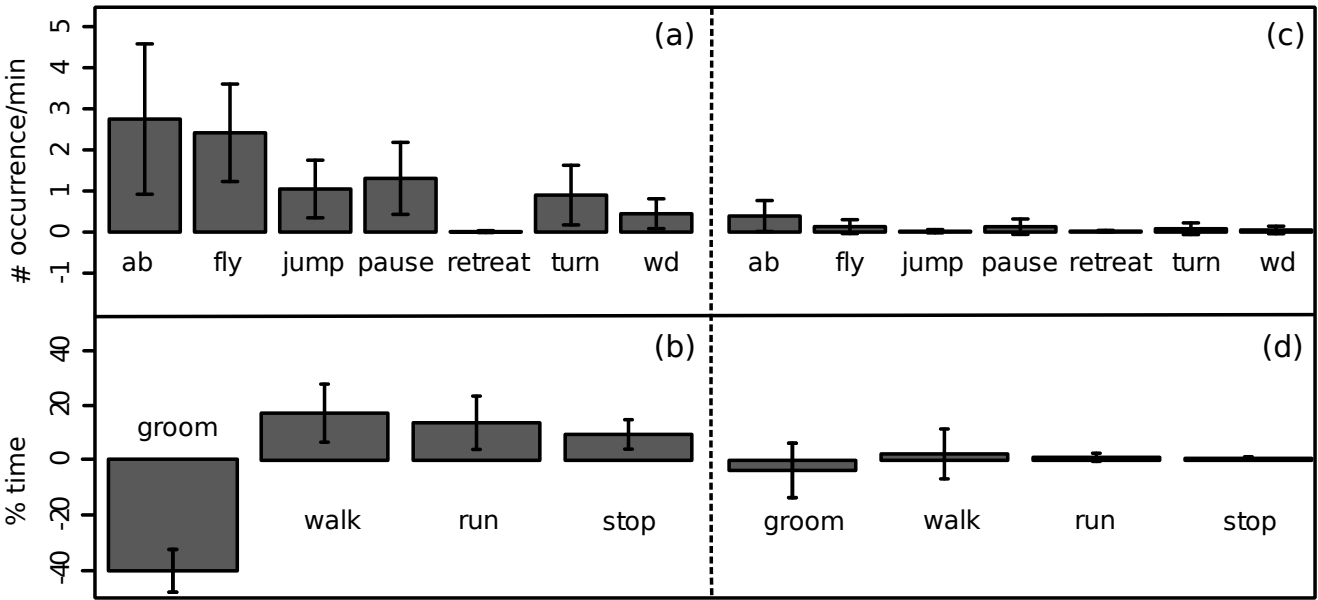
Figure 2

Fruit flies increase overall activity levels in the presence of jumping spiders **(a)** and **(b)** but not in the presence of mantids **(c)** and **(d)**. Plots (a) and (b) show change in mean number of occurrences per minute of each behavioural state as a result of the addition of a predator. Plots (c) and (d) show mean change in percentage of total time spent in a given behavioural state caused by the addition of a predator. On the left of the dotted line, behavioural changes correspond to the presence of a spider whereas on the right of the dotted line, behavioural differences are due to the presence of a juvenile praying mantid. Error bars are ± 95% CI.

241 both in their baseline activity levels as well as in their propensities to respond to jumping

242 spiders. While most individuals reduced their grooming activity in the presence of

243 predators, the degree to which they did so varied substantially (Figure 3a and 3b).

244 Interestingly, we did not see significant sex specific differences in either frequencies of

245 occurrence (Supplement b Figure S3) or proportion of time allocated (Supplement b

246 Figure S1) to the majority of measured behaviours (But see S3 panels "pause" and

247 "turn"). There was an overall negative correlation between the amount of time

248 individuals spent grooming before and after the addition of the spiders (Table 2). That is,

249 on average, individuals who were more active prior to the addition of the spider reduced

250 their activity to a greater extent in the presence of the spider.

251 To visualise the temporal associations among behavioural sequences, we

252 constructed transition matrices (Supplement b Tables S1, S2, S5 and S6) and transition

253 probability diagrams for all pairs of behaviours in the absence (Supplement b Figure S7)

254 and presence (Figure 4a) of predators. In response to jumping spiders, transitions among

255 behaviours are somewhat more dispersed (with many connections between behaviours),

256 suggesting that there is weak temporal association between fruit fly behaviours. Indeed

257 these qualitative conclusions are supported based on the Z-scores. In the absence of

258 spiders 8 possible transitions were significant (after controlling for multiple comparison,

259 Supplement b Table S1), while 13 transitions were significant in the presence of the

260 spider (Supplement b Table S2). Most of these differences were due to the increase in

261 behaviours potentially involved with anti-predation activity (i.e. flight, abdominal

262 lifting). However, while the results of the log-linear analysis (across the whole transition

263 frequency matrix) supported the dependence of current behavioural states on the previous
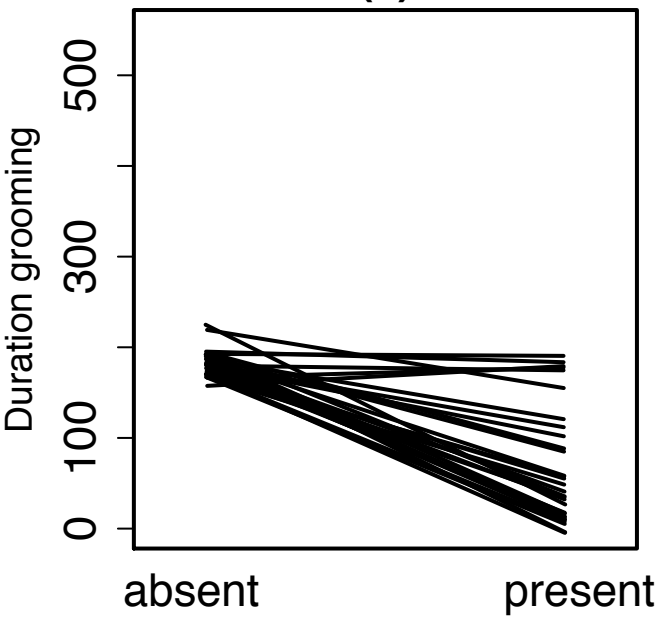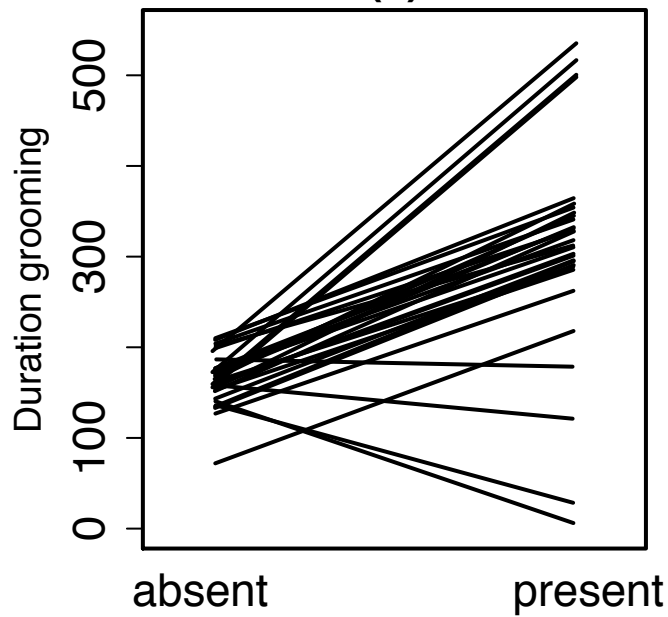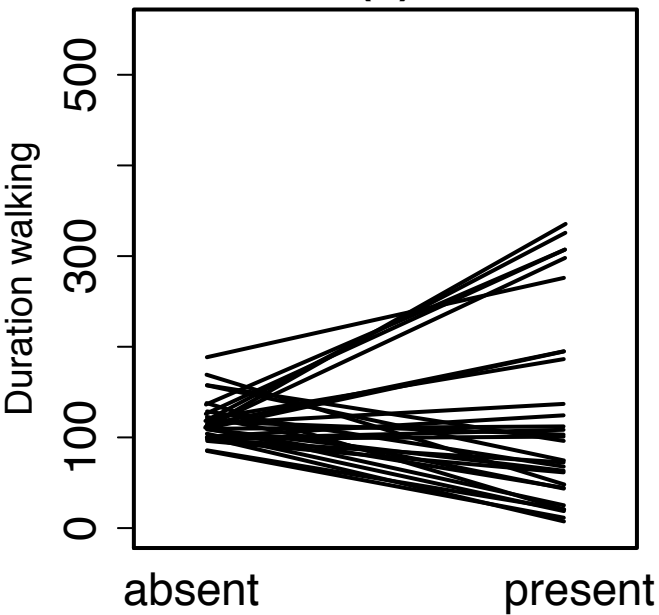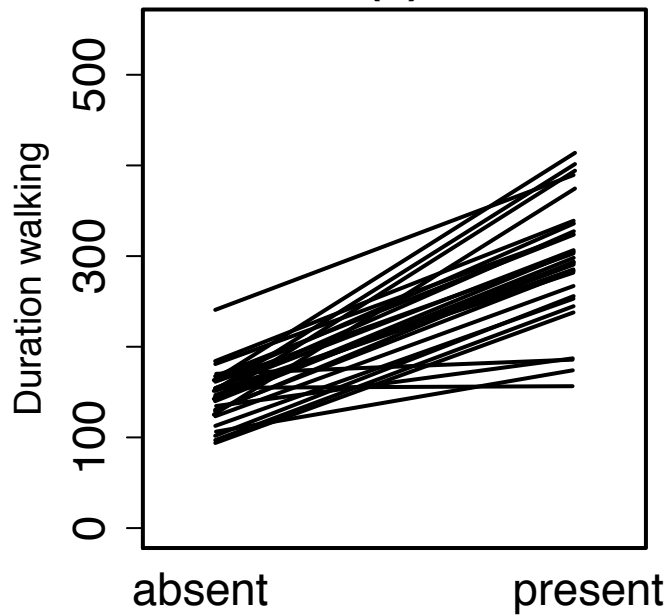
Table 2 Individual flies show negative correlations between behavioural states before and after the introduction of a predator. There is considerable variation among individuals in time spent performing specific behaviours (i.e. walking and grooming), with and without predators. However, there is a strong negative correlation within individuals for time spent before and after introduction of the predator. That is, individuals who spend more time performing a specific behaviour prior to the addition of a predator, reduce that behaviour to an even greater amount (than the average for the sample) once the predator is introduced. The one exception is for grooming for the mantid trials. Diagonals of the table contain the standard devation (mean of the posterior distribution) for individual behavioural responses (95% CIs in paratheses) from the random effects of the models. Above the diagonal are covariances between predictors (and CIs in parantheses). Below the diagonal are correlation coefficients for the covariances between the predictors.

Grooming, Spider

	Intercept	Pred.State	Time
Intercept	89.2 (62.0, 115.3)	-68.2 (-88.7, -34.1)	26.4 (14.0, 36.0)
Pred.State	-0.84	61.7 (18.8, 84.5)	-14 (-25.5, 17.1)
Time	0.75	-0.3	10.4 (4.4, 14.9)

Walking, Spider

	Intercept	Pred.State	Time
Intercept	81.9 (43.3, 109.8)	-60.2 (-89.5, 27.5)	25.6 (-8.8, 36.7)
Pred.State	-0.66	67.3 (0.36, 98.5)	11.5 (-22.2, 29.2)
Time	0.62	0.15	13 (5.6, 18.2)

Grooming, Mantid

	Intercept	Pred.State	Time
Intercept	122.8 (59.6, 175)	-20.2 (-117, 106)	46.0 (-16.9, 74.5)
Pred.State	-0.05	60.5 (0.13, 100.2)	-18.3 (-47.9, 34.0)
Time	0.8	-0.26	21.5 (2.6, 33.8)

Walking, Mantid

	Intercept	Pred.State	Time
Intercept	144.8 (86.2, 198)	-100.3 (-162.6, 38.8)	63.2 (31.6, 90.3)
Pred.State	-0.86	80.5 (0.21, 139.3)	-45.2 (-76.4, 19.6)
Time	0.94	-0.86	29.4 (11.3, 43.5)

Figure 3

Inter individual behavioural variation in respone to predators is present in natural populations. Reaction norms visualize how each individual fruit fly responded to the introduction of a spider (plannels a and b) or a mantid (pannels c and d) into the assay chamber. Measures are in seconds. Each line corresponds to reponse of one individual. Estimates are derived from the predicted values for each individual from the mixed models.
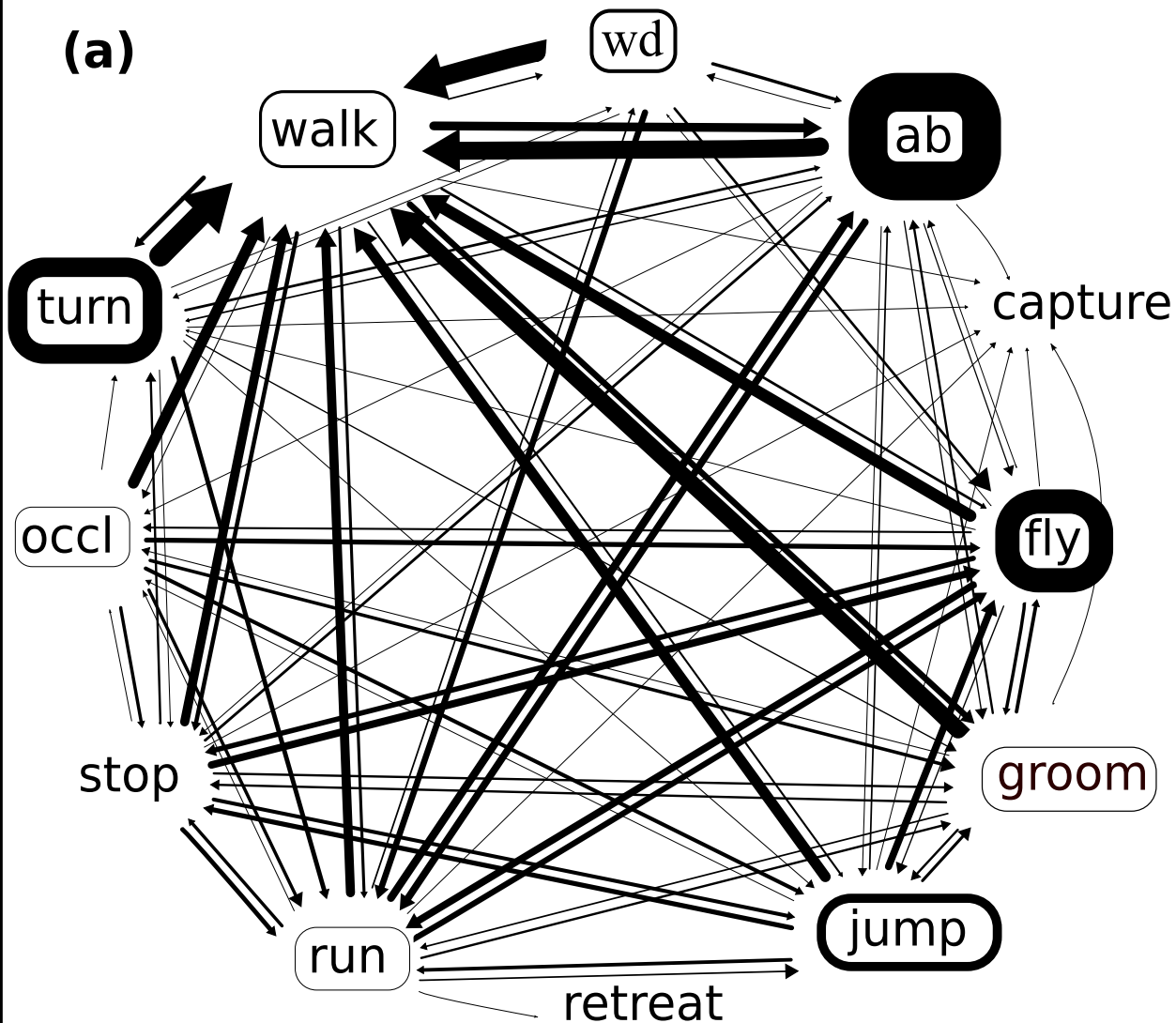
264   state (resid df=71, deviance=632, p < 0.001), the inclusion of predator status did not

265   influence this dependence (resid df = 71, deviance = 59, p = 0.8).

266

267   ***Flies perform a previously undescribed retreat behaviour in response to mantids***

268   In contrast to their behaviour in the presence of jumping spiders, the presence of a

269   juvenile praying mantid had minimal influence on *D. melanogaster*'s locomotory activity

270   (Figure 1, Supplement a). Time spent grooming, walking, running and stopping was

271   largely unaffected by the presence of a juvenile praying mantid (Figure 2d, Supplement b

272   Figures S1 and S2). Similarly, the presence of a mantid did not influence the frequency at

273   which *D. melanogaster* tended to perform most instantaneous behaviours (Figure 2a,

274   Supplement b Figure S4). However, as was observed with spiders, flies performed the

275   abdominal lifting behaviours (ab) at a significantly higher rate in the presence of a

276   juvenile praying mantid (Supplement b Figure S4). In addition, upon encounter with a

277   mantid, half of the individuals (15/30) performed a previously undescribed reversal

278   behaviour (Supplement c video 3), which we term "retreat". As with the zebra spiders,

279   we saw no significant sex specific differences in response to mantids.

280   Although the presence of a mantid had a small effect on fly behaviour, flies did

281   vary considerably in their grooming and walking activities. Indeed, the among-individual

282   variability in proportion of time spent grooming and walking is greater in magnitude in

283   the presence of the mantids than spiders (Figure 3). The results from the mixed model

284   did not demonstrate as strong support for the negative co-variation between an

285   individual's activity (walking, grooming) before and after the addition of the mantid

286   (Table 2).

(a)

(b)

wd

walk

ab

capture

turn

occl

fly

stop

groom

run

jump

retreat
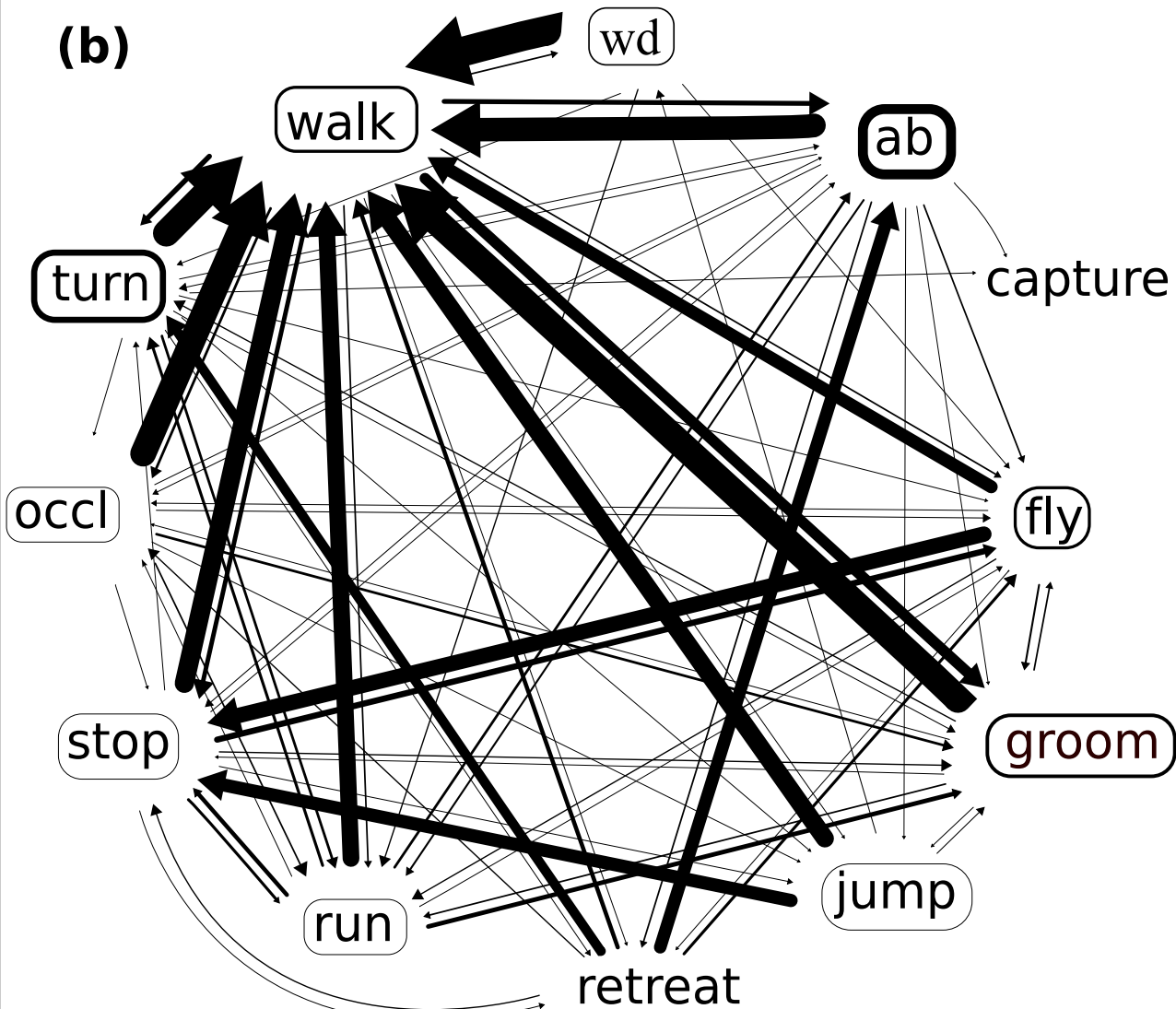
Figure 4

Spiders and mantids had different effects on the temporal associations between pairs of *D. melanogaster* behaviours. a) A diagram representing probability of transitioning from one fly behavioural state to the other in the presence of a zebra jumping spider. b) A diagram representing probability of transitioning from one fly behavioural state to the other in the presence of a juvenile praying mantid. Thickness of arrows indicate transition probability between the two behaviours. The arrowhead points to the behaviour being transitioned to. Thickness of the box around behavioural state (groom, run, occl, retreat, stop and walk) indicate the mean proportion of total time spent in that behaviour, whereas thickness of the box around behavioural events (fly, jump, turn, wd, ab) indicates mean number of occurrences per minute of that behaviour. To reduce the complexity of the web we combined the behaviours "pause" with the behaviour "stop". Behavioural transitions that occurred less than 10 times have not been shown in the figure.

287   Transition matrices and transition probability diagrams (Supplement b Figure S7,

288   Figure 4b and Tables S3, S4, S7 and S8) show patterns of temporal association among

289   behaviours. In response to juvenile mantids, the transitions diagram is less dispersed than

290   that in the presence of jumping spiders (Figure 4), suggesting that the degree of

291   association between behaviours in the presence of mantids is more extreme. While most

292   behaviours (abdominal lift, fly, groom, jump, run, stop, and turn) tend to transition to

293   walking, we also see stronger associations between other pairs of behaviours. For

294   example: after performing the retreat behaviour, fruit flies often either abdominal lift or

295   turn, and flight is often followed by stopping. These observations are supported by the

296   findings that in the absence of mantids, 12 transitions showed significant deviations from

297   expectations (Supplement b, Table S4). In comparison, in the presence of mantids 23

298   transitions showed a significant deviation from expected values (Supplement b, Table

299   S4). Interestingly, as with the spiders the log-linear model supports the non-independence

300   of behavioural states (resid df=71, deviance=1054, p <0.001), but not for the additional

301   influence of predator state on this non-independence (resid df = 71, deviance = 72,

302   p=0.4).

303

304   **DISCUSSION**

305   Prey organisms can alter their behaviour to reduce the likelihood of detection,

306   capture or encounter with a predator [5]. For example, when predators are present,

307   ground squirrels dedicate more time to vigilance behaviours (like scanning for a predator,

308   see [51]) and some aquatic insects spend more time in refuges [52]. These changes in

309   behaviour may alter the use of resources, and potentially the fitness of an organism.

310   However, the nature and intensity of non-consumptive effects of a predator on its prey

311   are a function of several predator specific factors, one of which is the predator's hunting

312   mode [10]. Predator hunting mode, i.e., the set of behavioural strategies that a predator

313   employs to pursue and capture its prey [8-10] can be an important determinant of a prey

314   organism's anti-predatory behavioural response [10,11]. In this study, we describe the

315   anti-predatory behavioural repertoire of a natural population of *Drosophila melanogaster*

316   in response to predation by the zebra jumping spider (*Salticus scenicus)* and juvenile

317   Chinese praying mantids (*Tenodera aridifolia sinensis*) that, among other characteristics,

318   differ in hunting mode. While we discuss our findings with respect to hunting mode

319   differences, we recognize that other attributes differing among the predators may

320   contribute to the observed differences in prey behavioural repertoires. However, as our

321   experimental design was meant to minimize the effects of many possible confounding

322   factors (e.g. time of day, temperature, humidity) it seems likely that, in part, our results

323   reflect hunting mode differences.

324   In response to active hunters (those that constantly patrol for prey), we predicted

325   that fruit flies would increase their overall activity levels (including flight) in order to

326   maintain maximum distance from the predator at all times [11]; To reduce the likelihood

327   of an encounter with an ambush predator however (i.e., a predator that only attacks when

328   a prey organism wanders in to its strike zone), we predicted that *D. melanogaster* would

329   respond by decreasing locomotory activities. Our results, however, were only partially in

330   line with these predictions. While the actively hunting jumping spiders induce a clear

331   increase in overall activity, we found the presence of juvenile mantids- our ambush

332   predators- to have minimal influence on fruit fly activity levels (Figure 2, Supplement b

333   Figure S2). It has been previously argued that ambush predators might be a predictable

334   source of threat to prey organisms [53,54] reviewed in [10] as opposed to the diffuse and

335   variable threat imposed by active hunters [11]. Therefore, it is perhaps surprising that

336   fruit flies show a stronger behavioural response to the threat of active hunters (zebra

337   jumping spiders) (see [55]). However, our predictions are based on studies on a

338   grasshopper and its two predatory spider species that differ in hunting mode. Given that

339   selection pressures faced by adult diptera are different from those experienced by

340   grasshoppers (orthoptera), such predictions may not be generalizable. Several factors

341   including body size and dispersal patterns may contribute to this difference. Many

342   species of jumping spiders, including *S. scenicus,* are often seen in the natural habitat of

343   *D. melanogaster* (personal observations of A.P., C.P. and I.D.), and are likely to be

344   ecologically relevant predators of *Drosophila*. Mantids however, are rarely found in

345   areas where fruit flies are abundant (personal observations of A.P. and I.D.), at least in

346   Eastern North America. Therefore, it is likely that fruit flies, having experienced a longer

347   evolutionary history with small jumping spiders, are better able to recognize these spiders

348   as a threat. In addition, the disturbance created by a constantly patrolling zebra spider

349   may be partly responsible for the increased activity levels seen in *D. melanogaster* (either

350   due to actual mechanical disturbance or because flies are able to detect moving objects

351   quicker than stationary ones). In this study, we are unable to tease apart the effects of

352   evolutionary recognition versus constant mechanical disturbance on the differences in

353   flies' activity levels. Further experimentation with harmless but constantly moving

354   heterospecifics (such as field crickets) or immobilized active hunters might be useful in

355   addressing these issues.

356   We also identified a number of (to our knowledge) undescribed behaviours of *D.*

357   *melanogaster,* potentially relating to its interactions with predators. The behaviour we

358   called "stopping" (Table 1) was observed numerous times after a direct (but failed) attack

359   by a spider (Supplement 3 video 1). While *D. melanogaster* will spend time without any

360   ambulatory activity (walking, running), they are almost always observed to be active

361   (generally grooming) during these periods. However, when fruit flies performed the

362   stopping behaviour, there was a complete lack of movement on the part of the fly, even

363   when video was viewed at a few frames/second. When a fruit fly was "stopped", the

364   spider had to search for the fly, irrespective of the physical proximity between the spider

365   and the fly. In salticids, while the principal eyes have high spatial acuity [34], secondary

366   eyes are primarily used to detect moving objects [56]. Because salticids are unable to

367   accommodate by changing the shape of their lens, they need to extensively sample their

368   visual field to see details in object shape and form [34,55,58]. Scanning for prey by such

369   sampling is likely a slow process unless guided by the motion sensing peripheral eyes,

370   giving motionless prey the advantage of staying hidden (at least for a few seconds) while

371   in plain sight of their salticid predator. Thus, *D. melanogaster* may be using the

372   "stopping" behaviour as a potential mechanism to reduce the likelihood of detection by

373   the spider.

374   Additionally, in the presence of both predators, *D. melanogaster* substantially

375   increase the frequency at which it performed abdominal lifts. To our knowledge,

376   abdominal lifting has not been described in *D. melanogaster* literature before and may be

377   relevant in an anti-predatory context. While studying courtship behaviours in female *D.*

378   *melanogaster*, Lasbleiz el al described two behaviours perhaps similar to the abdominal

379   lifting described here: abdominal drumming and abdominal extension [57]. Abdominal

380   drumming (described as "quickly repeated vertical movements of the abdomen which is

381   tapped on the substrate") was only seen in males during courtship display, and abdominal

382   extensions (described as " abdomen raised by 15-30 degrees") were also closely

383   associated with courtship. Because abdominal lifting was often directed at a predator or

384   followed a failed predatory encounter, we suspect abdominal lifting to be different from

385   abdominal extensions and abdominal drumming, and with a possibly anti-predatory

386   function. We speculate that if abdominal lifting is indeed anti-predatory, it could function

387   in one of several possible ways. First, abdominal lifting may be a signal of prey condition

388   directed at the predator as a form of pursuit deterrence, comparable to stotting in the

389   Thomson's gazelle [60]. Second, because *D. melanogaster* are often surrounded by

390   conspecifics, abdominal lifting may be a means though which one fly warns its

391   conspecifics of the presence of a potential threat (similar in function to fin flicking in

392   tetras, [61]). Finally it may be an indication of some sort of physiological priming of the

393   fly in preparation for a fight-or-flight response. Determining whether it is a specific anti-

394   predator behaviour, as well as the details of its function need to be a focus of future work.

395   In response to the juvenile praying mantids, half of the fruit flies we observed

396   (15/30) performed a reverse walking behaviour which we have called "retreat", where the

397   flies walked in reverse, away from the predator (supplement c, video 3). This was often

398   (but not always) interspersed with the abdominal lifting behaviour. Phenomenologically,

399   this behaviour may be similar to that described in Bidaye et al [62], where the authors

400   identified neurons that upon activation changed walking direction in *D. melanogaster*.

401   Bidaye et al's reverse walking behaviour, appears to be a smooth and continuous

402   behaviour, whereas the "retreat" was often discontinuous and accompanied by abdominal

403   lifting. If the two "retreat" behaviours are related, the observed disassociation between

404   retreat and abdominal lifting as well as its continuous nature (in [62]) may be a function

405   of how the neurons were perturbed.

406   We also investigated how the presence of the different predators may influence

407   non-random associations among behaviours. We observed that in the presence of both

408   predators there was an increase in the number of behavioural transitions that deviated

409   from expectations under independence (from 12 to 23 with the mantid, and 8 to 13 with

410   the spider). Despite this, the log-linear model (analysing the whole transition frequency

411   matrix) did not support the influence of predator state on the frequencies of transitions.

412   This may be partly due to the relatively modest sample sizes (in terms of both individuals

413   and transitions among behaviours). Further work is necessary to validate and extend this

414   sequential analysis.

415   While we show that there are some predator hunting-mode specific behavioural

416   differences in *D. melanogaster's* anti-predator response, we reiterate two important

417   caveats. First, although the primary distinction between the zebra jumping spider and

418   juvenile Chinese praying mantids as predators is their hunting-mode, other factors

419   between these species (for example, size, colour, odour) may also influence differences in

420   fruit fly behaviours. Replicating the observations with other predator pairs that differ in

421   hunting-mode is necessary to confirm hunting-mode's influence on anti-predatory

422   repertoires. Secondly, our assay chambers are an artificial environment and do not

423   resemble the conditions under which *D. melanogaster* face predators in the wild. Due to

424   the nature of our assay chamber, *D. melanogaster* were unable to employ behavioural

425   strategies that may reduce encounters with predators (e.g., utilizing a refuge). Therefore

426   we were only able to describe the capture-deterrence repertoire of *D. melanogaster*

427   behaviour. We believe that our study is a necessary first step to describing and

428   documenting the complete anti-predatory behavioural repertoire of *D. melanogaster* and

429   we foresee future work to be conducted in a modified chamber, under more "natural"

430   conditions. Doing so will allow us to take this premier model genetic system and make it

431   into an ecological model as well.

432

433   ***ACKNOWLEDGEMENTS***

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1   **Supplemental material**

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3

4

5   **Supporting information**

6

7   *Differential response to spiders versus mantids*

8   Because spider and mantid population densities vary by season, we had to temporally

9   segregate the spider assays from the mantid assays. We conducted all spider observations

10   between October and December 2012 and all the mantid observations between March and May

11   2013. Comparing time allocation and frequencies of occurrences in the predator absent state

12   between the two predator treatments suggest that behavioural modifications were predator

13   induced, and not due to seasonal effects (Figure S5 and S6). Although the assays were carried

14   out under highly controlled conditions, to confirm that predator species-specific behavioural

15   differences were not confounded with seasonal differences in behaviour, we performed 6

16   additional assays (alternating between spider and mantid treatments) within the span of one

17   week. The control experiments show no evidence of confounding effects of season with *D.*

18   *melanogaster*'s anti-predator behavioural repertoire (Table S9, S11 and S12 below). Ethograms

19   are shown in Supplement a. Furthermore, to confirm that the disturbance we caused (to the assay

1

20   chamber) during the addition of a predator did not confound behavioural responses to the

21   predator, we did 3 "no predator" control assays. For these "no predator" controls, instead of

22   adding a predator to the arena, we caused a mild disturbance (~ to intensity of disturbance caused

23   while adding the predator) without actually adding any predator. We found that disturbance

24   caused during predator addition was not responsible for observed behavioural modifications

25   (Table S10 and S13). Finally, "no predator" controls also ruled our temporal differences in fruit

26   fly activity levels (Table S10 and S13)

27

28   **Supplemental figures**

29
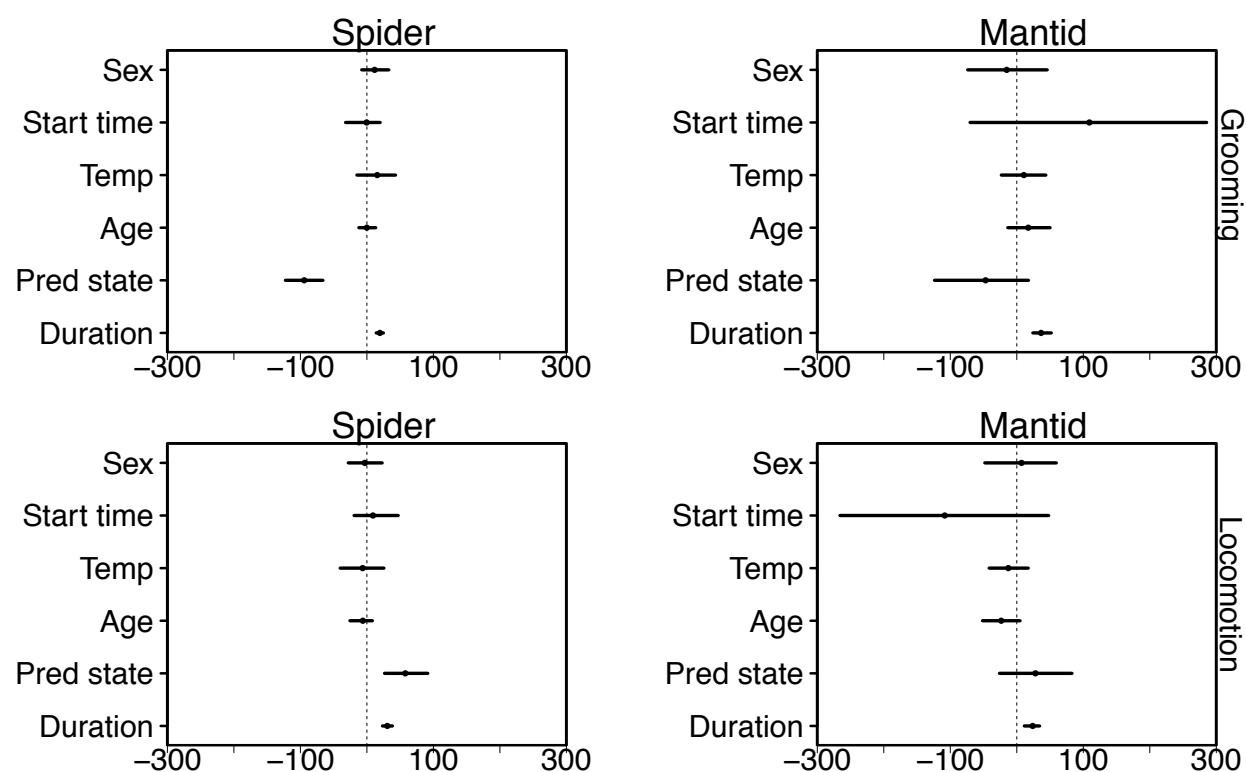
30   *Figure S1*

31   Addition of jumping spiders cause fruit flies to walk more and groom less, whereas the presence

32   of mantids show weaker, more variable (and not significant) changes in fruit fly activity levels.

2

33   Here we show coefficient plots from the output of mixed effects models using the package

34   MCMCglmm to visualize duration of two behaviours (grooming and locomotion) as a function

35   of predator state (present vs absent of spiders, left panels and mantids, right panels), total

36   duration of the assay, sex of the fly, start time of the assay, temperature in the room and age of

37   the fly. Estimates are in seconds. Error bars are ± 95% CI.

38
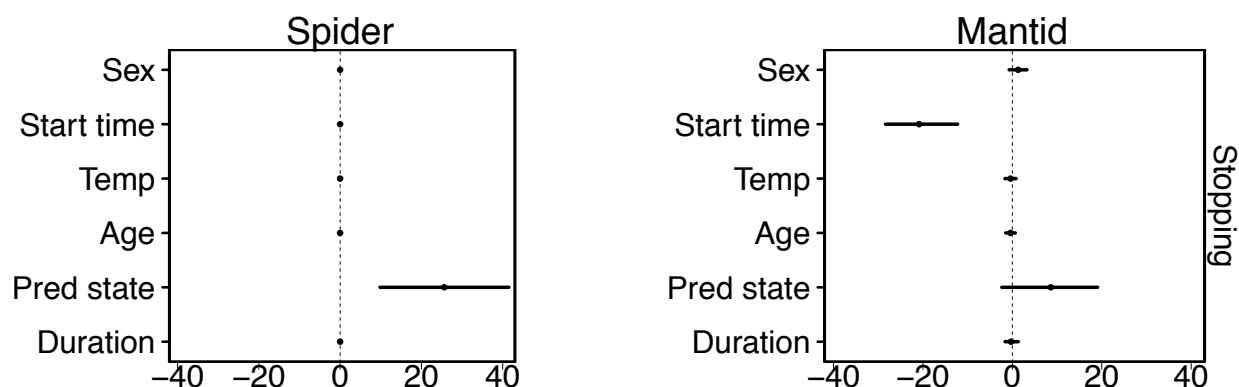
39   *Figure S2*

40   Fruit flies "stop" significantly longer in the presence of spiders (left panel), and to a much lesser

41   extent (and not significantly) in the presence of mantids (right panel). Here we have coefficient

42   plots made from the output of mixed effects models using the package MCMCglmm to visualize

43   duration of "stopping" as a function of predator state (present vs absent), total duration of the

44   assay, sex of the fly, start time of the assay, temperature in the room and age of the fly. Estimates

45   are in seconds. Error bars are ± 95% CI. Although assays were performed between 9 am and 12

46   pm each day, start time for the mantid assays significantly affected the total time that flies spent

47   "stopping".

48

3

49
50 *Figure S3*
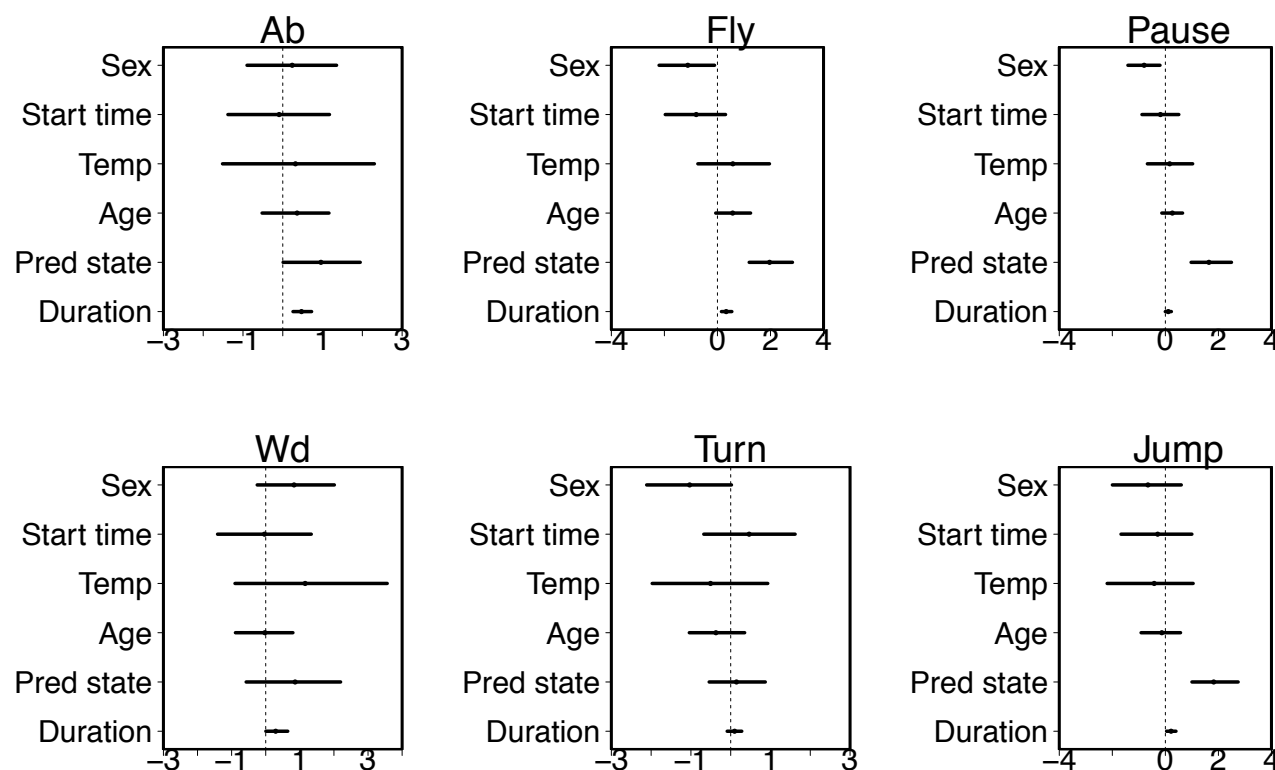
51 In the presence of spiders, fruit flies increased the frequency with which they performed flights,

52 pauses and jumps. Here we show coefficient plots made from the output of mixed effects models

53 using the package MCMCglmm to visualize duration of each individual behavioural event (ab,

54 fly, pause, wd, turn and jump) as a function of predator state (present vs absent of a spider), total

55 duration of the assay, sex of the fly, start time of the assay, temperature in the room and age of

56 the fly. All estimates are scaled to number of events per minute. Error bars are ± 95% CI.

57

58

59
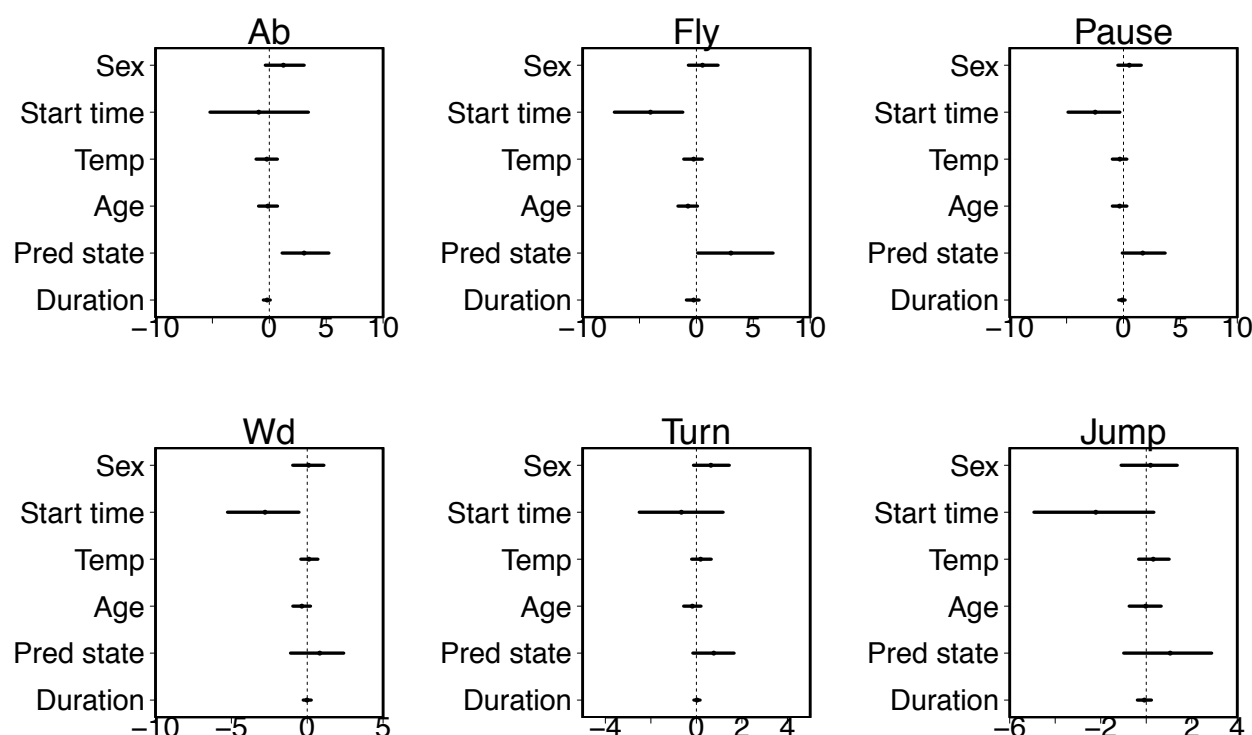
60   *Figure S4*

61   Fruit flies performed abdominal lifts are a higher frequency in the presence of a juvenile mantid.

62   Here we show coefficient plots made from the output of mixed effects models using the package

63   MCMCglmm to visualize duration of each individual behavioural event (ab, fly, pause, wd, turn

64   and jump) as a function of predator state (present vs absent of a mantid), total duration of the

65   assay, sex of the fly, start time of the assay, temperature in the room and age of the fly. All

66   estimates are scaled to number of events per minute. Error bars are ± 95% CI. Although assays

67   were performed between 9 am and 12 pm each day, start time for the mantid assays significantly

68   affected the frequency at which *D. melanogaster* performed the "Fly", "Wd" and "Jump"

69   behaviours.

70

5

71
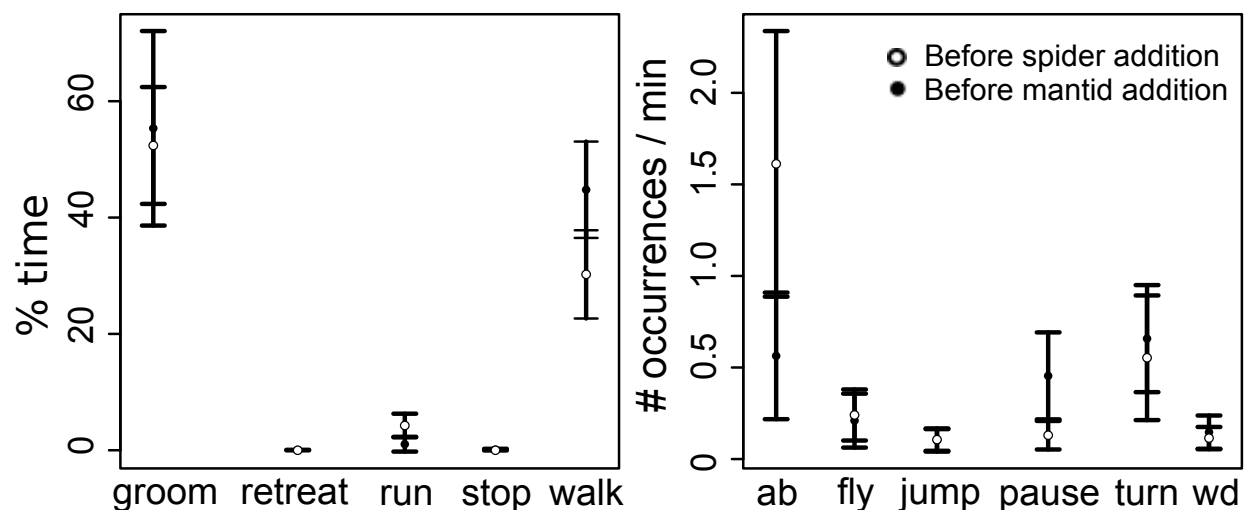
Figure S5

Hunting mode induced behavioural differences in fruit fly behaviours were not confounded with seasonal effects. Here we show percentage time spent in each behavioural state (left) and number of occurrences per minute for each behavioural event (right) as measured for individual fruit flies before the addition of a spider (white circles) and before the introduction of a mantid (black circles) into the chamber. Error bars are ± 2 * SEs. Overlapping error bars suggest that there was minimal effect of season on the behavioural repertoire of fruit flies.

79

80
81 *Figure S6*
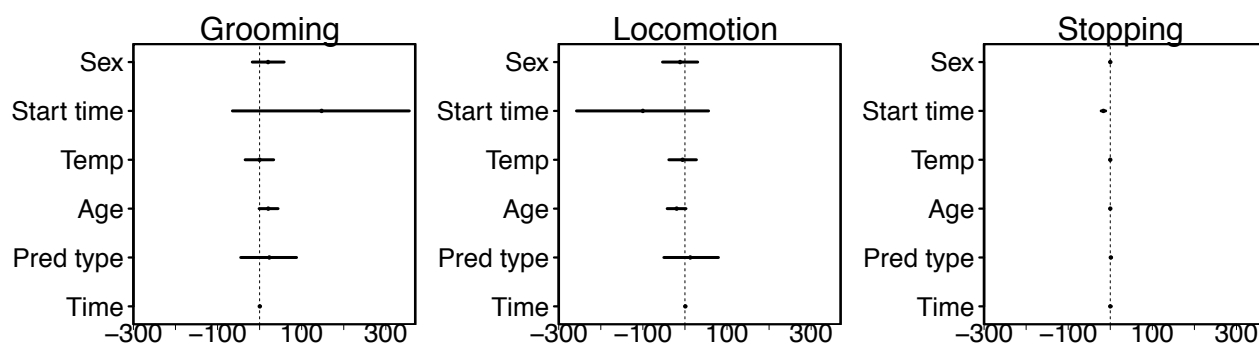
82   Seasonal differnces in fruit fly behaviours did not confound behavioural differences induced by

83   difference hunting-modes. Flies measured before the addition of a spider did not differ in

84   behaviour from flies measured before the addition of a mantid.
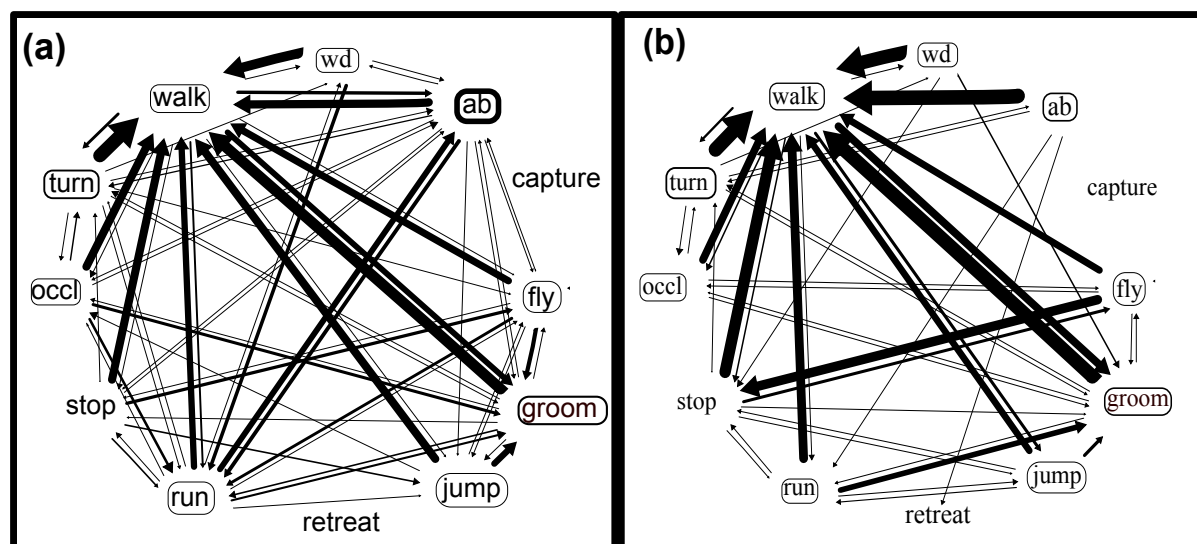
85

86

87   *Figure S7*

88   **a)** A diagram representing probability of transitioning from one fly behaviour to the other

89   when individuals were measured before the addition of a spider **b)** A diagram representing

90   probability of transitioning from one fly behaviour to the other for individuals measured

91   before the addition of a juvenile mantid. Thickness of arrows indicates transition

92   probability between the two behaviours. The arrowhead points to the behaviour being

93   transitioned to. Thickness of the box around behavioural state (groom, run, occl, retreat,

94   stop and walk) indicate the mean proportion of total time spent in that behaviour, whereas

95   thickness of the box around behavioural events (fly, jump, turn, wd, ab) indicates mean

96   number of occurrences per minute of that behaviour. To reduce the complexity of the web

7

97   we combined the behaviours "pause" with the behaviour "stop". Behavioural transitions

98   that occurred less than 10 times have not been shown in the figure.

99

100   **Supplemental tables**

101

102   **Table S1** Transition frequency matrix when a spider was present in the chamber. Each row

103   represents the number of times one behaviour (row name) transitioned to another behaviour

104   (column name). Numbers in blue represent transitions that occurred more often that expected

105   under a model of independence, whereas numbers in red are transitions that occurred less often

106   than expected (see methods).

Spider

Behavior	ab	capture	fly	groom	jump	retreat	run	stop	occl	turn	walk	wd	Total
ab	0	2	8	9	7	0	45	6	5	10	112	7	211
capture	0	0	0	0	0	0	0	0	0	0	0	0	0
fly	4	2	0	15	7	0	34	25	10	1	58	1	157
groom	11	3	16	0	10	0	8	11	5	7	77	2	150
jump	4	2	19	8	0	0	9	13	2	0	28	0	85
retreat	1	0	0	0	0	0	0	0	0	0	0	0	1
run	43	4	33	13	9	1	0	18	3	4	51	9	188
stop	9	3	32	10	16	0	19	0	1	9	38	0	137
occl	3	0	6	4	5	0	4	4	0	1	14	0	41
turn	8	1	0	4	3	0	14	2	0	0	66	2	100
walk	122	7	36	87	26	0	42	60	14	63	0	24	481
wd	5	0	4	0	0	0	9	0	0	1	29	0	48
Total	210	24	154	150	83	1	184	139	40	96	473	45	1599

107

108

109   **Table S2** Transition frequency matrix before a spider was added to the chamber. Each row

110   represents the number of times one behaviour (row name) transitioned to another behaviour

111   (column name). Numbers in blue represent transitions that occurred more often that expected

112   under a model of independence, whereas numbers in red are transitions that occurred less often

113   than expected (see methods)s.

8

No spider

Behavior	ab	capture	fly	groom	jump	retreat	run	stop	occl	turn	walk	wd	Total
ab	0	0	2	16	1	0	30	1	2	10	65	2	129
capture	0	0	0	0	0	0	0	0	0	0	0	0	0
fly	1	0	0	9	1	0	6	2	0	0	15	0	34
groom	7	0	11	0	8	0	18	1	5	10	122	0	182
jump	0	0	1	7	0	0	0	0	1	0	9	0	18
retreat	0	0	0	0	0	0	0	0	0	0	0	0	0
run	38	0	5	14	1	0	0	4	2	1	42	3	110
stop	1	0	4	0	2	0	2	0	0	1	10	0	20
occl	2	0	0	6	0	0	5	0	0	3	16	0	32
turn	4	0	1	5	0	0	2	0	3	0	72	1	88
walk	74	0	8	126	5	0	42	13	18	64	0	12	362
wd	1	0	0	0	0	0	4	0	0	0	13	0	18
Total	128	0	32	183	18	0	109	21	31	89	364	18	993

114

115

116 **Table S3** Transition frequency in the presence of a juvenile praying mantid. Each row represents

117 the number of times one behaviour (row name) transitioned to another behaviour (column name).

118 Numbers in blue represent transitions that occurred more often that expected whereas numbers in

119 red are transitions that occurred less often than expected.

Mantid

Behavior	ab	capture	fly	groom	jump	retreat	run	stop	occl	turn	walk	wd	Total
ab	0	1	9	2	1	9	10	5	4	6	127	0	174
capture	0	0	0	0	0	0	0	0	0	0	0	0	0
fly	0	0	0	7	0	1	5	60	2	0	50	0	125
groom	0	0	17	0	3	0	18	3	5	13	305	1	365
jump	0	0	0	1	0	0	0	13	0	0	18	1	33
retreat	9	0	2	0	0	0	0	1	1	7	3	0	23
run	11	0	3	18	0	0	0	17	1	12	68	0	130
stop	4	0	45	9	4	5	21	0	0	5	139	0	232
occl	1	0	3	7	1	0	5	1	0	0	73	0	91
turn	4	1	2	6	1	3	15	0	1	0	164	0	197
walk	145	2	43	326	23	5	51	132	73	150	0	49	999
wd	0	0	1	0	0	0	2	0	0	1	47	0	51
Total	174	4	125	376	33	23	127	232	87	194	994	51	2420

120

121

122 **Table S4** Transition frequency matrix before a juvenile mantid was added to the chamber. Each

123 row represents the number of times one behaviour (row name) transitioned to another behaviour

9

124 (column name). Numbers in blue represent transitions that occurred more often that expected

125 whereas numbers in red are transitions that occurred less often than expected.

No mantid

Behavior	ab	capture	fly	groom	jump	retreat	run	stop	occl	turn	walk	wd	Total
ab	0	0	0	0	0	1	1	0	0	1	31	0	34
capture	0	0	0	0	0	0	0	0	0	0	0	0	0
fly	0	0	0	1	0	0	0	19	1	0	11	0	32
groom	0	0	9	0	1	0	4	1	3	12	212	0	242
jump	0	0	0	3	0	0	1	4	0	0	6	0	14
retreat	1	0	0	0	0	0	0	1	1	0	0	0	3
run	0	0	0	9	1	0	0	1	0	0	15	0	26
stop	0	0	14	1	1	0	1	0	0	1	54	0	72
occl	0	0	1	2	0	0	0	0	0	1	55	0	59
turn	2	0	0	2	0	0	0	0	4	0	79	0	87
walk	31	0	8	236	10	1	19	45	48	71	0	21	490
wd	0	0	0	2	0	0	0	1	0	0	18	0	21
Total	34	0	32	256	13	2	26	72	57	86	481	21	1080

126

127

128 **Table S5** Transition probability from one behaviour (row name) to the other (column name) in

129 the presence of a zebra jumping spider. Transition probabilites are obtained by dividing each

130 transition frequeny (see table S1) between a pair of behaviours by the total number of times a

131 given behaviour was performed (row sums in table S1).

Spider

Behavior	ab	capture	fly	groom	jump	retreat	run	stop	occl	turn	walk	wd
ab	0.00	0.01	0.04	0.04	0.03	0.00	0.22	0.03	0.02	0.05	0.55	0.03
capture	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
fly	0.03	0.01	0.00	0.10	0.04	0.00	0.22	0.16	0.06	0.01	0.37	0.01
groom	0.07	0.02	0.11	0.00	0.07	0.00	0.05	0.07	0.03	0.05	0.52	0.01
jump	0.05	0.02	0.22	0.09	0.00	0.00	0.11	0.15	0.02	0.00	0.33	0.00
retreat	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
run	0.24	0.02	0.18	0.07	0.05	0.01	0.00	0.10	0.02	0.02	0.28	0.05
stop	0.07	0.02	0.23	0.07	0.12	0.00	0.14	0.00	0.01	0.07	0.28	0.00
occl	0.07	0.00	0.15	0.10	0.12	0.00	0.10	0.10	0.00	0.02	0.34	0.00
turn	0.08	0.01	0.00	0.04	0.03	0.00	0.14	0.02	0.00	0.00	0.67	0.02
walk	0.27	0.02	0.08	0.19	0.06	0.00	0.09	0.13	0.03	0.14	0.00	0.05
wd	0.10	0.00	0.08	0.00	0.00	0.00	0.19	0.00	0.00	0.02	0.60	0.00

132

133

134   **Table S6** Transition probability from one behaviour (row name) to the other (column name)

135   before a zebra jumping spider was introduced into the arena. Transition probabilites are obtained

136   by dividing each transition frequeny (see table S1) between a pair of behaviours by the total

137   number of times a given behaviour was performed (row sums in table S1).

No spider

Behavior	ab	capture	fly	groom	jump	retreat	run	stop	occl	turn	walk	wd
ab	0.00	0.00	0.02	0.13	0.01	0.00	0.24	0.01	0.02	0.08	0.51	0.02
capture	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
fly	0.03	0.00	0.00	0.26	0.03	0.00	0.18	0.06	0.00	0.00	0.44	0.00
groom	0.04	0.00	0.06	0.00	0.04	0.00	0.10	0.01	0.03	0.05	0.67	0.00
jump	0.00	0.00	0.06	0.39	0.00	0.00	0.00	0.00	0.06	0.00	0.50	0.00
retreat	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
run	0.36	0.00	0.05	0.13	0.01	0.00	0.00	0.04	0.02	0.01	0.39	0.03
stop	0.05	0.00	0.20	0.00	0.10	0.00	0.10	0.00	0.00	0.05	0.50	0.00
occl	0.06	0.00	0.00	0.19	0.00	0.00	0.16	0.00	0.00	0.09	0.50	0.00
turn	0.05	0.00	0.01	0.06	0.00	0.00	0.02	0.00	0.03	0.00	0.83	0.01
walk	0.21	0.00	0.02	0.36	0.01	0.00	0.12	0.04	0.05	0.18	0.00	0.03
wd	0.06	0.00	0.00	0.00	0.00	0.00	0.22	0.00	0.00	0.00	0.72	0.00

138

139

140   **Table S7** Transition probability from one behaviour (row name) to the other (column name) in

141   the presence of a juvenile praying mantid. Transition probabilites are obtained by dividing each

142   transition frequeny (see table S1) between a pair of behaviours by the total number of times a

143   given behaviour was performed (row sums in table S1).

Mantid

Behavior	ab	capture	fly	groom	jump	retreat	run	stop	occl	turn	walk	wd
ab	0.00	0.01	0.05	0.01	0.01	0.05	0.06	0.03	0.02	0.03	0.73	0.00
capture	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
fly	0.00	0.00	0.00	0.06	0.00	0.01	0.04	0.48	0.02	0.00	0.40	0.00
groom	0.00	0.00	0.05	0.00	0.01	0.00	0.05	0.01	0.01	0.04	0.84	0.00
jump	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.41	0.00	0.00	0.56	0.03
retreat	0.39	0.00	0.09	0.00	0.00	0.00	0.00	0.04	0.04	0.30	0.13	0.00
run	0.08	0.00	0.02	0.14	0.00	0.00	0.00	0.13	0.01	0.09	0.52	0.00
stop	0.02	0.00	0.19	0.04	0.02	0.02	0.09	0.00	0.00	0.02	0.60	0.00
occl	0.01	0.00	0.03	0.08	0.01	0.00	0.05	0.01	0.00	0.00	0.80	0.00
turn	0.02	0.01	0.01	0.03	0.01	0.02	0.08	0.00	0.01	0.00	0.83	0.00
walk	0.15	0.00	0.05	0.34	0.02	0.01	0.05	0.14	0.08	0.16	0.00	0.05
wd	0.00	0.00	0.02	0.00	0.00	0.00	0.04	0.00	0.00	0.02	0.92	0.00

Table S8 Transition probability from one behaviour (row name) to the other (column name) before a juvenile praying mantid was introduced into the arena. Transition probabilites are obtained by dividing each transition frequeny (see table S1) between a pair of behaviours by the total number of times a given behaviour was performed (row sums in table S1).

No mantid

Behavior	ab	capture	fly	groom	jump	retreat	run	stop	occl	turn	walk	wd
ab	0.00	0.00	0.00	0.00	0.00	0.03	0.03	0.00	0.00	0.03	0.91	0.00
capture	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
fly	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.59	0.03	0.00	0.34	0.00
groom	0.00	0.00	0.04	0.00	0.00	0.00	0.02	0.00	0.01	0.05	0.88	0.00
jump	0.00	0.00	0.00	0.21	0.00	0.00	0.07	0.29	0.00	0.00	0.43	0.00
retreat	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.33	0.00	0.00	0.00
run	0.00	0.00	0.00	0.35	0.04	0.00	0.00	0.04	0.00	0.00	0.58	0.00
stop	0.00	0.00	0.19	0.01	0.01	0.00	0.01	0.00	0.00	0.01	0.75	0.00
occl	0.00	0.00	0.02	0.03	0.00	0.00	0.00	0.00	0.00	0.02	0.93	0.00
turn	0.02	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.05	0.00	0.91	0.00
walk	0.07	0.00	0.02	0.50	0.02	0.00	0.04	0.10	0.10	0.15	0.00	0.04
wd	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.05	0.00	0.00	0.86	0.00

Table S9 Proportion of time spent in a given behavioural state by each individual fruit fly before and after introducing a treatment (i.e., a disturbance, spider or mantid) to the assay chamber.

Treatment	Individual	Grooming		Walking		Running		Stopping		Retreat	
		Absent	Present	Absent	Present	Absent	Present	Absent	Present	Absent	Present
Disturbance	1	0.820	0.728	0.180	0.265	0.000	0.005	0.000	0.000	0.000	0.000
	2	0.590	0.265	0.338	0.658	0.000	0.009	0.044	0.015	0.000	0.000
	3	0.264	0.219	0.658	0.685	0.048	0.036	0.013	0.052	0.000	0.000
Spider	1	0.528	0.490	0.359	0.394	0.022	0.011	0.000	0.003	0.000	0.000
	2	0.116	0.000	0.844	0.861	0.000	0.086	0.000	0.053	0.000	0.000
	3	0.706	0.181	0.262	0.368	0.010	0.076	0.000	0.347	0.005	0.002
Mantid	1	0.419	0.764	0.474	0.217	0.008	0.005	0.061	0.014	0.000	0.000
	2	0.673	0.075	0.327	0.785	0.000	0.014	0.000	0.093	0.000	0.006
	3	0.684	0.963	0.309	0.037	0.000	0.000	0.000	0.000	0.000	0.000

Table S10 Number of occurrences per minute of each behavioural event before and after the introduction of a treatment (i.e., a disturbance, spider or mantid) to the assay chamber.

Treatment	Individual	Pause		Turn		Ab		Wd		Fly		Jump	
		Absent	Present	Absent	Present	Absent	Present	Absent	Present	Absent	Present	Absent	Present
Disturbance	1	0.000	0.006	0.000	0.006	0.000	0.003	0.000	0.000	0.000	0.002	0.000	0.003
	2	0.003	0.013	0.003	0.013	0.060	0.023	0.003	0.005	0.082	0.027	0.000	0.005
	3	0.000	0.002	0.000	0.002	0.027	0.052	0.003	0.010	0.045	0.090	0.018	0.016
Spider	1	0.009	0.013	0.000	0.005	0.043	0.046	0.006	0.000	0.006	0.003	0.000	0.000
	2	0.020	0.018	0.032	0.045	0.012	0.000	0.012	0.027	0.048	0.252	0.000	0.000
	3	0.015	0.011	0.003	0.011	0.026	0.090	0.000	0.002	0.018	0.041	0.003	0.000
Mantid	1	0.052	0.017	0.016	0.006	0.031	0.002	0.008	0.002	0.070	0.020	0.003	0.000
	2	0.005	0.060	0.005	0.012	0.023	0.111	0.000	0.003	0.000	0.076	0.000	0.006
	3	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table S11 Coefficients from a linear model (lm) for control individuals meansured before and after the addition of a spider. While estimate of posterior means are similar to those of the main spider dataset, Due to low sample sizes, CIs are large.

Grooming			
Coefficient	Posterior mean	Lower CI	Upper CI
Intercept	181.88	-23.34	387.10
Predator state	-75.38	-377.82	227.05
Duration	0.45	-0.34	1.24
Locomotion			
Intercept	156.76	37.32	276.21
Predator state	17.27	-158.75	193.29
Duration	0.24	-0.22	0.70

163

164

165 **Table S12** Coefficients from a linear model (lm) for control individuals meansured before and

166 after the addition of a mantid. Estimate of posterior means are similar to those of the main mantid

167 dataset, but due to low sample sizes, CIs large.

Grooming			
Coefficient	Posterior mean	Lower CI	Upper CI
Intercept	134.79	-1399.38	1668.96
Predator state	341.56	-2661.10	3344.23
Duration	-0.80	-12.76	11.17
Locomotion			
Intercept	345.69	-919.99	1611.36
Predator state	-323.02	-2800.19	2154.15
Duration	1.64	-8.24	11.51

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170 **Table S13** Coefficients from a linear model (lm) for control individuals meansured before and after

171 a disturbance. Despite low sample sizes, it is clear that disturbance had minimal effect on fruit fly

172 behaviours.

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Grooming			
Coefficient	Posterior mean	Lower CI	Upper CI
Intercept	200.00	60.11	339.81
Disturbance	0.00	-197.78	197.78
Duration	2.38	-0.53	5.29
Locomotion			
Intercept	132.30	-0.28	264.84
Disturbance	0.00	-187.47	187.47
Duration	-1.05	-3.81	1.70

174

175 **Links to videos describing novel behaviours**

176 1) Abdominal Lifting http://dx.doi.org/10.6084/m9.figshare.1185638

177 2) Stopping Behaviour http://dx.doi.org/10.6084/m9.figshare.1185639

178 3) Retreat http://dx.doi.org/10.6084/m9.figshare.1185640