How predator hunting-modes affect prey behaviour: Capture deterrence in *Drosophila melanogaster*

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Links to videos (also available in supplement)

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

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

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

ABSTRACT

Hunting mode, the distinct set of behavioural strategies that a predator employs while hunting, can be an important determinant of the prey organism's behavioural response. However, few studies have considered how a predator's hunting mode influences anti-predatory behaviours of a prey species. Here we document the influence of active hunters (zebra jumping spiders, *Salticus scenicus*) and ambush predators (Chinese praying mantids, *Tenodera aridifolia sinensis*) on the capture deterrence anti-predatory behavioural repertoire of the model organism, *Drosophila melanogaster*. We hypothesized that *D. melanogaster* would reduce overall locomotory activity in the presence of ambush predators, and increase activity with active hunters. First we observed and described the behavioural repertoire of *D. melanogaster* in the presence of the predators. We documented three previously undescribed behaviours- abdominal lifting, stopping and retreat- which were performed at higher frequency by *D. melanogaster* in the presence of predators, and may aid in capture deterrence. Consistent with our predictions, we observed an increase in the overall activity of *D. melanogaster* in the presence of jumping spiders (active hunter). However, counter to our prediction, mantids (ambush hunter) had only a modest influence on activity. We also observed considerable intra and inter-individual variation in response to both predator types. Given these new insights into *Drosophila* behaviour, and with the genetic tools available, dissecting the molecular mechanisms of anti-predator behaviours may now be feasible in this system.

INTRODUCTION

Predation, a ubiquitous selective force, gives rise to and determines the nature of defensive traits in prey populations (Edmunds, 1974; Juliano & Gravel 2002; Goslin & Rodd, 2007; Langerhans, 2007; Lima & Dill, 1990; Sansom, Lind & Cresswell, 2009). Predator hunting-modes, i.e., the set of behaviours that predators employ to pursue and capture their prey (Schoener, 1971; Huey & Pianka 1981; Preisser, Orrock & Schmitz 2007), have been shown to induce distinct prey responses (Schmitz, 2008) that in turn influence the productivity of ecological communities. In habitats dominated by active hunters there is lower species evenness and higher above-ground net primary productivity compared to habitats dominated by ambush hunters (Schmitz, 2008). The authors suggest the observed differences in prey productivity to be driven by hunting mode specific trade-offs between foraging and seeking refuge. Although studies often describe the effects of predators on prey traits (i.e. DeWitt, Robinson & Wilson, 2000; Reznick, Butler & Rodd, 2001; Relyea, 2001), it is rare for the role of predator hunting-mode to be explicitly considered.

Here we investigate segregating differences in the anti-predatory behavioural repertoire of the fruit fly, *Drosophila melanogaster*, in response to two predator species differing in hunting modes. Based on (Schmitz, 2008), we predicted that fruit flies, in the presence of a familiar predator, would exhibit hunting-mode specific modifications in activity levels. We used *D. melanogaster* because, although it is one of the most well-studied model organisms, there is a relative paucity of information regarding *D. melanogaster's* natural history, ecology and behaviour, including habitat, food resources, and natural enemies (but see Reaume & Sokolowski, 2006; David & Capy, 1988; Turelli

64 & Hoffmann, 1991; Schmidt, et al. 2005; Fleury et al. 2004; Wilfert & Jiggins, 2014;
65 Stephan & Li, 2006). While anti-predator behaviours are well studied as targets of
66 selection in prey (Juliano & Gravel, 2002; Stoks, McPeek & Mitchell, 2003; Magurran et
67 al. 1992), the genetic bases of such behaviours are seldom investigated. Given the range
68 of genetic and genomic tools available for *D. melanogaster,* along with its complex
69 behavioural repertoire and suitability for experimental evolution, understanding the anti-
70 predatory behaviours persisting in a natural population of the fruit fly brings us one step
71 closer to deciphering the molecular mechanisms for anti-predator behaviours.

72 Previous work has examined the effects of natural enemies on population and
73 community structures of *Drosophila spp*. Worthen (1989) studied the effects of predation
74 by rove beetles (staphylinids) on the coexistence of three mushroom-feeding *Drosophila*
75 species, and Escalante & Benado (1990) showed that ant predators regulate population
76 densities of wild *D. starmeri* (cactophillic fruit fly). In *D. melanogaster per se*, the role of
77 parasites in influencing larval and adult behaviours has been extensively studied (Milan,
78 Kacsoh & Schlenke, 2012; Kacsoh et al., 2013; Polak & Starmer, 1998). Despite this
79 literature, we know little about the predators of *D. melanogaster* adults in the wild, nor
80 the nature of anti-predatory behaviours segregating in natural populations.

81 We documented the influence of two predators, the zebra jumping spider (*Salticus*
82 *scenicus*) and juvenile Chinese praying mantids (*Tenodera aridifolia sinensis*) on the
83 capture-deterrence behaviours of *D. melanogaster* individuals derived from a wild-caught
84 population. The zebra spider is an active hunter, locating prey visually (with an extensive
85 visual field attained by antero-medially positioned simple eyes) (Dill, 1975; Horner,
86 Stangl & Fuller, 1988). Mantids are generally ambush predators, waiting for prey to enter

their attack range (Prete, Klimek & Grossman, 1990). Despite numerous differences, zebra spiders and juvenile Chinese mantids are similar in two relevant ways. First, both species primarily detect prey visually (Forster, 1979; Harland, Jackson, Macnab, 1999; Jackson, & Blest, 1982; Prete 1999) and are likely incapable of depth perception when their prey item is motionless (Prete, 1999; Freed, 1984). Second, small adult diptera account form a substantial proportion of the diet of both predators in the wild (Iwasaki, 1998; Okuyama, 2007).

Based on the findings of Schmitz (2008), we predicted that fruit flies, in the presence of a familiar predator, would exhibit hunting-mode specific modifications in activity levels. To maximize distance from the actively hunting spider, our prediction was that flies would increase their overall activity levels, whereas, to reduce the probability of encountering a stationary threat (the mantid, an ambush predator), we expected flies to decrease overall activity.

Under controlled laboratory conditions, we documented the behaviours of individual adult *D. melanogaster* with and without the two predator species. Our results suggest that in the presence of zebra spiders, *D. melanogaster* increases its overall locomotory activity, performs a distinct "stopping" behaviour and increases the performance of a newly described abdominal lifting behaviour (the function of which is as of yet unknown). Counter to our prediction though, *D. melanogaste*r's locomotion, and most other behaviours are not substantially altered in the presence of mantids. However, upon direct encounter with a mantid, many individuals of *D. melanogaster* perform (a previously undescribed) retreat behaviour- a response not generally elicited by jumping spiders. Furthermore we observe considerable intra- and inter-individual variation in

110 response to predators. We discuss our results in terms of conditionally expressed
111 behaviours as they relate to predator hunting mode, co-evolutionary history of predators
112 and prey, and in terms of broadening our understanding of the behavioural ecology of *D.*
113 *melanogaster*.
114
115 **METHODS**
116 ***Drosophila Population and Culture Conditions***
117 The *Drosophila melanogaster* population used in this study originated from a
118 natural population at Fenn Valley Vineyards in Fennville, Michigan (GPS coordinates:
119 42.57, -86.14) during the summer of 2010. A lab population (henceforth referred to as
120 FVW) was initiated from this collection using the progeny of over 500 single-pair
121 matings of field caught *D. melanogaster* as well as wild caught males. This design
122 allowed us to screen out the sympatric congener, *D. simulans,* which was present in our
123 collections at a frequency of about 5%. Screening involved setting up single pair mating
124 in vials and discarding all lines with *D. simulans*-like genital morphology. After
125 screening, ~1500 individuals were placed into cage (32.5cm^3, BugDorm BD43030F) to
126 establish the FVW population. The population is currently maintained in this cage at an
127 adult density ~ 3000 individuals in a room maintained at 23^{0}C (+/- 1^{0}C), and 40-70% RH.
128 Adults were allowed to lay eggs in 10 bottles with 50-60 ml of a standard yeast-cornmeal
129 food for 2-3 days. These bottles were then removed and kept in a Percival incubator
130 (Model: I41VLC8) at 24^{0}C and 65% RH throughout the larval stages. All flies and larvae
131 were maintained in a 12 hr light/dark cycle with lights on at 08:00 hours.

132 For the experiments, pupae were collected 24 hours before they emerged as
133 adults. Pupae were removed from bottles using forceps and individual pupae were placed
134 into 1.5 ml microcentrifuge tubes. Each tube was pre-filled with ~ 0.5 ml of yeast-
135 cornmeal food and its cap was punctured for gas exchange. Upon emergence, adult flies
136 were sexed visually without anesthesia and housed in these tubes in the incubator until
137 needed for behavioural assays. Age of flies used in behaviour analysis was 3-7 days. By
138 using socially naïve flies in our assays, we were able to establish a consistent baseline of
139 social experience among all individuals, allowing us to eliminate the potentially
140 confounding influence of variation in social experience on behaviour that is well-
141 documented in *Drosophila* (Yurkovic et al., 2006; Levine, 2004; Krupp et al., 2008;
142 Lefranc et al., 2001; Chabaud et al., 2009).
143
144 ***Spiders***
145 *S. scenicus* individuals were collected throughout the spring/summer of 2012 on
146 the campus of Michigan State University. Spiders were housed individually in vials in a
147 room maintained at 23^0C (+/- 1^0C) and 30-50% RH and fed ~5 *D. melanogaster* a week.
148 Prior to use in behavioural assays, spiders were starved for at least 48 hours. Each spider
149 was used in only a single behavioural assay.
150
151 ***Mantids***
152 Mantid egg cases were both collected near the campus of Michigan State
153 University as well as ordered from Nature's Control (Medford, Oregon). Mantid egg
154 cases were stored at 4^0C and transferred to 25^0C and 70% RH for hatching. Given the

substantial changes in mantid body size across moults (Iwasaki, 1990), only first instar nymphs were used for experiments. Prior to behavioural assay, mantids were starved for at least 24 hours and each mantid was used only once.

Behavioural Assays

All assays were performed 1-4 hours after the incubator lights came on in the morning (08:00). Behavioural assays were recorded with an Aiptek AHD H23 digital camcorder attached to a tripod under a combination of natural and fluorescent light that is present in the room wherein the FVW population and spiders are maintained. For each predator (spiders and mantids), we recorded the behaviour for each of 15 male and 15 female socially naïve, virgin flies (collected as described above). We used a chamber constructed from the bottom of a 100 x15mm petri dish inverted on top of a glass plate with a sheet of white paper beneath to maximize the visibility of flies and predators.

For each assay, an individual fly was aspirated into the chamber and allowed to acclimate for 5 minutes. After this acclimation period, flies were recorded for 5 minutes. A single spider or mantid was then introduced to the chamber and behaviours were recorded for an additional 10 minutes or until capture. The chamber was washed with 10-30% ethanol and rinsed with reverse osmosis water after each assay to remove olfactory cues.

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Behaviours Recorded

All *Drosophila* behaviours were categorized and analysed as either "states" or "events". Behavioural states have measurable duration and are mutually exclusive with

other states (e.g. individuals cannot simultaneously walk and run). Behavioural events are discrete behaviours that occur instantaneously and are also mutually exclusive with each other (e.g. turning versus jumping) but not always mutually exclusive with behavioural states. For example, an individual could perform a wing display (event) while simultaneously walking (state), but it could not jump (event) while simultaneously running (state). In this study we treated flying as an event because the structure of the experimental chamber constrained flight duration. Attempted flight by *D. melanogaster* could result in landing due to contact with a wall of the petri dish. We also recorded when a fly was not visible (occluded) to the observers analysing video. We recorded a total of 6 discrete events and 5 behavioural states in *D. melanogaster* in response to predation by spiders and mantids (Table 1). In order to interpret an individual fly's behaviour in the context of predatory encounters, we designated two keys to describe the location of the predator in regard to its interactions with the fly. As flies might alter their behaviour when a predator is within striking distance, we recorded predator location based on whether or not it was within striking distance of the fly (~ 5mm from the spider/mantid, also see *Spider location*/ *Mantid location* in Figure 1).

Video Processing

Recorded behaviours were viewed with VLC media player (version 2.0.3) and analysed by two observers using a manual event recorder, JWatcher V1.0 software (Blumstein, 2006). One observer (A.P.) viewed each video and verbally announced the occurrence of behaviours while the other observer (C.P./ M.C.) recorded the occurrence

200 of these behaviours with JWatcher. Because *Drosophila* anti-predatory behaviours are
201 often complex and occur rapidly, we analysed all videos at 0.5X speed.
202
203 ***Controlling for effects of season and disturbance***
204 We conducted experiments with spiders between October and December 2012 and those
205 with mantids from March and May 2013. To confirm that predator species-specific
206 behavioural differences were not confounded with seasonal differences in behaviour, we
207 performed 6 additional assays (alternating between spider and mantid treatments) within
208 the span of one week. Following a spider assay, the plates were wiped down with 30%
209 ethanol followed by a rinse with RO water before a mantid assay was conducted.
210 Additionally, the process of adding a predator to the arena invariably resulted in a
211 disturbance that likely startled the fly (unrelated to the presence of a predator). To
212 confirm that behaviours induced by this disturbance were not confounded with predator
213 induced behavioural differences, we performed 3 control assays. Here, after 5 minutes of
214 acclimatization without a predator (see above for more details), the arena containing the
215 fruit fly was disturbed gently (~ magnitude of disturbance caused by the addition of a
216 predator). For all controls, video processing and behaviours recorded were identical to
217 mantid and spider treatments described above. See Supplement b, S1 for a detailed
218 description of these control experiments and their results.
219
220 ***Data processing and statistical analysis***
221 A custom Python script was used to parse Jwatcher formatted data files into a
222 comma-separated-value (CSV) file for analysis in **R** (version 3.0.1).

To analyse the effects of predator state (i.e., presence or absence of predators) on the time dedicated to locomotory behavioural states, and number of occurrence for behavioural events, we fit generalized linear mixed effects models (using both `glmer` function in the lme4 package version 1.0-5, and the `MCMCglmm` function in the MCMCglmm package version 2.17) with predator state, total duration of assay with and without a predator (duration), sex, temperature and recording time as fixed effects, and individual by predator state and date as random effects . Formally, the model was:

$$\mathbf{y} \sim \beta_0 + \beta_1 PS + \beta_2 D + \beta_3 Ag + \beta_4 T + \beta_5 ST + \beta_6 Sx + \beta_7 + \boldsymbol{\varepsilon}$$

Where **y** is a vector of time spent in a behavioural state. β_1 is the regression coefficient for predator state, β_2 is for duration in each predator state, β_3 is for age of the fly, β_4 is for temperature, β_5 is for time at which assay was started, β_6 is for sex of the fly and β_7 is for date on which the assay was performed. We estimated random effects for individuals including variation in response to predator state and duration of assay, and we fit an independent random effect for date. Thus we fit a repeated effects (longitudinal) mixed effects model allowing for variation among individuals for the influence of predator presence and duration of assay where for the ith individual

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

and (independent of the above)

$\beta_7 \sim N(0, \sigma^2_j)$ where j = 1 … date

Preliminary analyses were inconsistent with the need to fit higher order interactions among fixed effects, so interaction terms were not considered further. The one exception was for "stopping" behaviour where individuals almost exclusively performed this in the presence of the predators. For the behavioural states (locomotion, grooming and stopping) we assumed normally distributed variation. For the counts of events (abdominal lift, jumping, etc) we used a log-link function and assumed the variation was poisson distributed. Estimation using both maximum likelihood (`lmer`) and simulating the posterior distribution (`MCMCglmm`) provided similar results for fixed effects, and generally for random effect components as well.

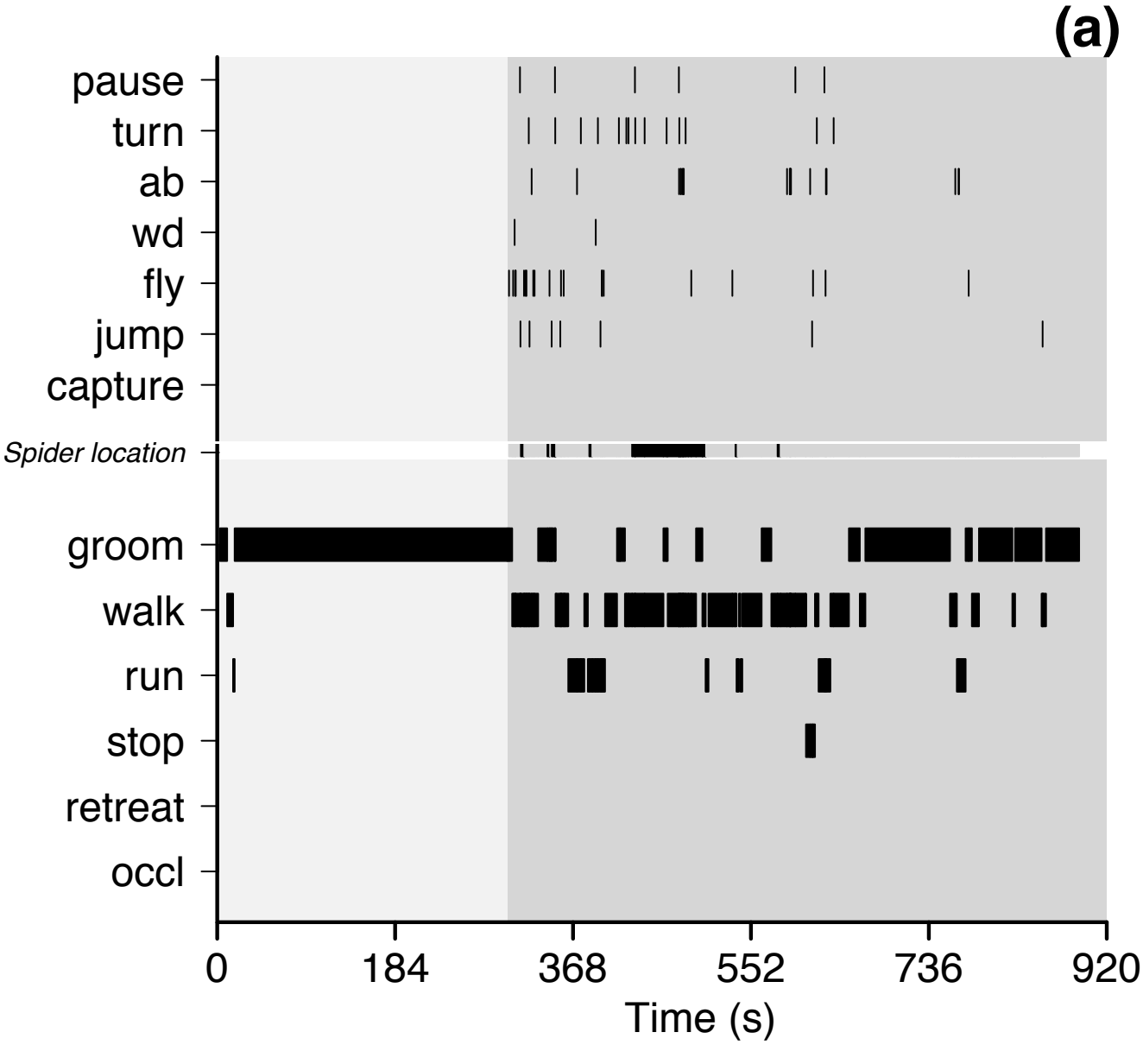
Among individual coefficients of variation were calculated by dividing the square root of the among individual variance component from the model by its respective fixed effect estimate (i.e. its "mean"). While confidence intervals were consistent for fixed effects, the intervals were more difficult to estimate given the complexities of the random effect structure of the model, and some caution is warranted for their interpretation.

To test for non-random associations in the temporal structure of behavioural patterns we constructed transition frequencies using the "msm" library (version 1.2) (Jackson, 2011) in **R**. To test for both for first order Markov processes between behaviours (transition probabilities), as well as the influence of predator presence on these transition probabilities, we fit log-linear models (assuming poisson distributed data) with the transition frequency matrices (Crawley, 2012) using `glm` in **R**. As advocated by (Crawley, 2012; Bakeman & Gottman, 1997) we fit a saturated log-linear model (with lag0, lag1 and predator state as the effects in the model) and tested the influence of

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)

268 deleting the terms (i.e. third order interaction) on change in deviance. We used modified
269 "Z-scores", adjusted using sequential Bonferroni to assess the deviation of particular cells
270 in the transition frequency matrix from expected values (assuming independence). For the
271 visual transition probability matrices, we combined the behavioural event "pause" with
272 the behavioural state "stop" because 1) we wanted to reduce the complexity of the matrix
273 and 2) the main difference between the two behaviours is that pause is instantaneous and
274 stop has duration. All transition diagrams were constructed in Inkscape (version 0.48.2,
275 Harrington, 2004-2005).
276
277 **RESULTS**
278 From pilot observations (not included in analysis), we (I.D., A.P. and C.P.)
279 catalogued and described *Drosophila melanogaster* behaviours observed in the presence
280 of a predator (Table 1). Among the behaviours listed in Table 1, abdominal lifting (ab,
281 supplement b, video 1) and retreat (supplement b, video 3), to our knowledge, have not
282 been previously described in *D. melanogaster* literature.
283
284 ***Flies perform a range of anti-predatory behaviours in response to a zebra spider***
285 To visualize each individual fruit fly's response to the presence of a zebra
286 jumping spider, we generated ethograms (see Figure 1a and Supplement a). For the two
287 predator states (spider present and spider absent) we measured the mean proportion of
288 time dedicated to each behavioural state, as well as the number of occurrences per minute
289 for each behavioural event. When a spider was present, *D. melanogaster* increased the
290 proportion of time it spent walking and running by 50% (95% CI: 21-79% increase)


(a)
pause
turn
ab
wd
fly
jump
capture
Spider location
groom
walk
run
stop
retreat
occl
0
184
368
552
736
920
Time (s)

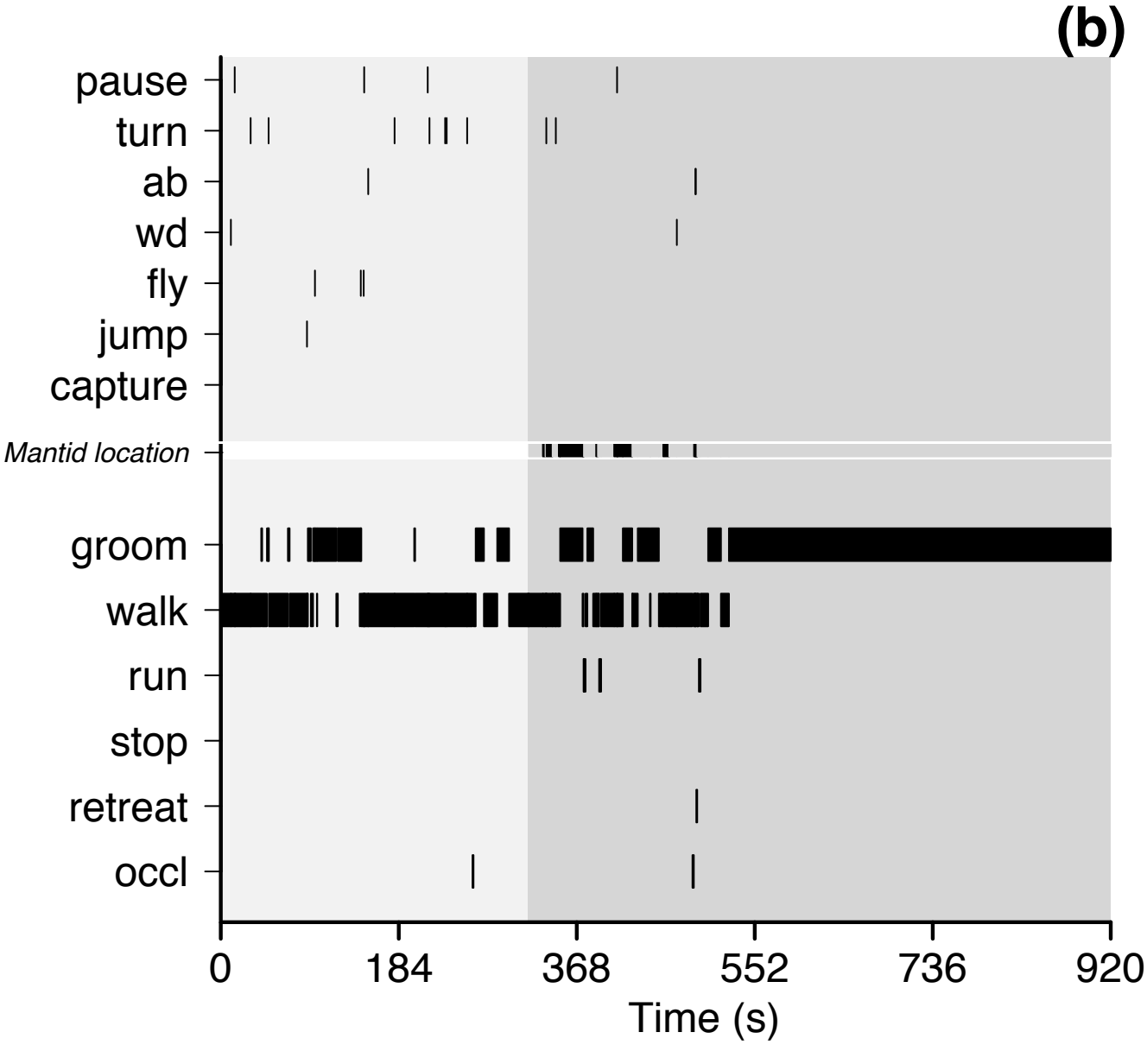

(b)
pause
turn
ab
wd
fly
jump
capture
Mantid location
groom
walk
run
stop
retreat
occl
0
184
368
552
736
920
Time (s)

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.

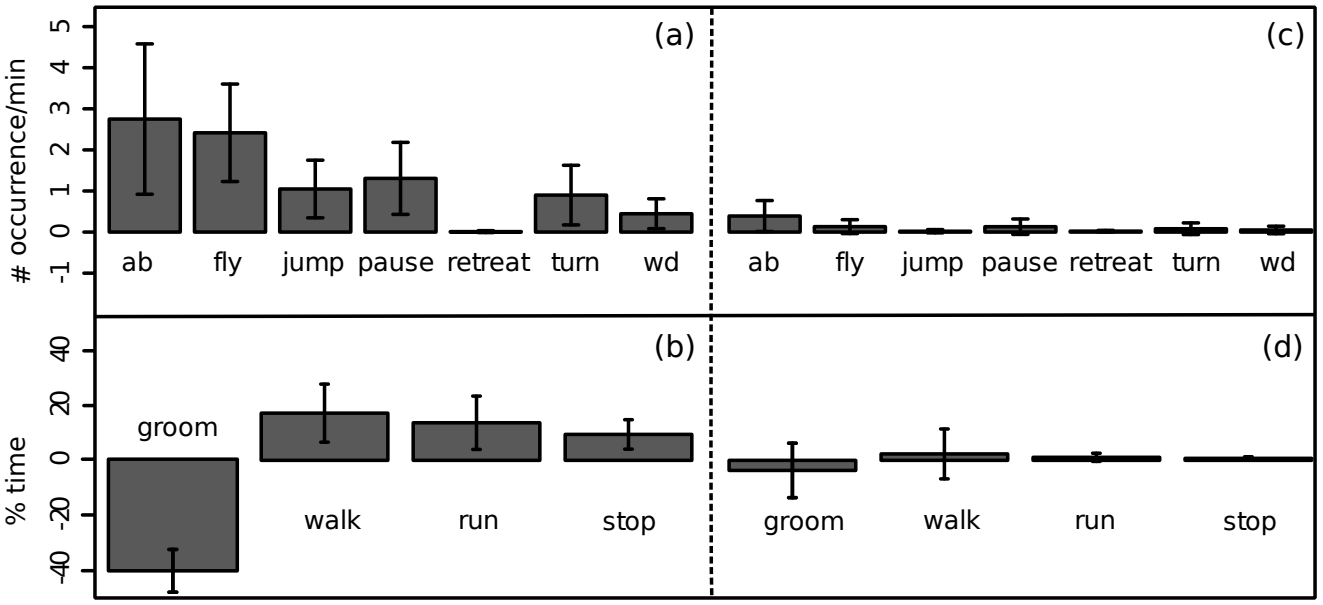

(a)
(c)
(b)
(d)
occurrence/min
% time
5
4
3
2
1
0
-1
40
20
0
-20
-40
ab
fly
jump
pause
retreat
turn
wd
groom
walk
run
stop

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.

291 while grooming 60% less (95% CI: 43-77% decrease). This is shown in Figure 2 and
292 Supplement b Figure S1 (treatment contrasts with 95% CI in figures are provided to
293 enable assessment of significance). While they were observed at low frequencies prior to
294 the addition of spiders, *D. melanogaster* substantially increased the frequency of pauses,
295 jumps and flights (per minute) in the presence of spiders (Figure 2, Supplement b Figure
296 S3). For instance the frequency of abdominal lifts increased from 1.51/minute to
297 4.0/minute (95% CI: 1.55-9.93), while jumping showed a 6.6X increase from 0.73/minute
298 to 4.82/minute (95% CI: 1.77-12.17). "Stopping"; a motionless state that likely aids in
299 capture deterrence (see Videos 2, Supplement b) was not performed by *D. melanogaster*
300 in the absence of spiders (Supplement b Figure S2). However in the presence of spiders,
301 the average total time spent "stopping" increased to ~25.8 seconds (95% CI: 10.1 - 41.7
302 seconds). When interacting with spiders, flies were only observed to perform the
303 "retreat" behaviour once (of 30 individuals). Interestingly, we did not see significant sex
304 specific differences in either frequencies of occurrence (Supplement b Figure S3) or
305 proportion of time allocate (Supplement b Figure S1) to the majority of measured
306 behaviours (But see S3 panels "pause" and "turn").
307 Given the design of our experiment, we were able to model the degree to which
308 individuals varied in their responses to the jumping spiders. Individuals varied greatly
309 both in their baseline activity levels as well as in their propensities to respond to jumping
310 spiders. The among-individual coefficient of variation for time spent grooming in the
311 absence of predators was 57.7% (40.1-74.2%). While most individuals reduced their
312 grooming activity in the presence of predators, the degree to which they did so varied
313 substantially, with the among individual coefficient of variation for the decrease being

314 67.8% (26.5-94.6%), as shown in Figure 3a. For walking, the among-individual
315 coefficient of variation was 80.3% (50-105%) in the absence of the spider, and 135% (1-
316 181%) for the magnitude of increase in the presence of the spider (Figure 3b).
317 Performance of the stopping behaviour by *D. melanogaster* in the presence of spiders
318 varied substantially among individuals, with the among-individual coefficient of variation
319 being 168% (95% CI: 123-214%). This is driven in part by the fact that 40% of
320 individual flies never performed stopping, even in the presence of the spider. There was
321 a negative correlation (-0.84), between the amount of time individuals spent grooming
322 before and after the addition of the spiders (Table 2). That is, on average, individuals who
323 were more active prior to the addition of the spider reduced their activity to a greater
324 extent in the presence of the spider. A similar negative correlation (-0.66) for among
325 individual activity for locomotion, was observed (Table 2).
326 To visualise the temporal associations among behavioural sequences, we
327 constructed transition matrices (Supplement b Tables S1, S2, S5 and S6) and transition
328 probability diagrams for all pairs of behaviours in the presence (Figure 4a) and absence
329 (Supplement b Figure S7) of spiders. In response to jumping spiders, transitions among
330 behaviours are somewhat more dispersed (with many connections between behaviours),
331 suggesting that there is weak temporal association between fruit fly behaviours. Indeed
332 these qualitative conclusions are supported based on the Z-scores. In the absence of
333 spiders 8 possible transitions were significant (after controlling for multiple comparison,
334 Supplement b Table S2), while 13 transitions were significant in the presence of the
335 spider (Supplement b Table S1). Most of these differences were due to the increase in
336 behaviours potentially involved with anti-predation activity (i.e. flight, abdominal

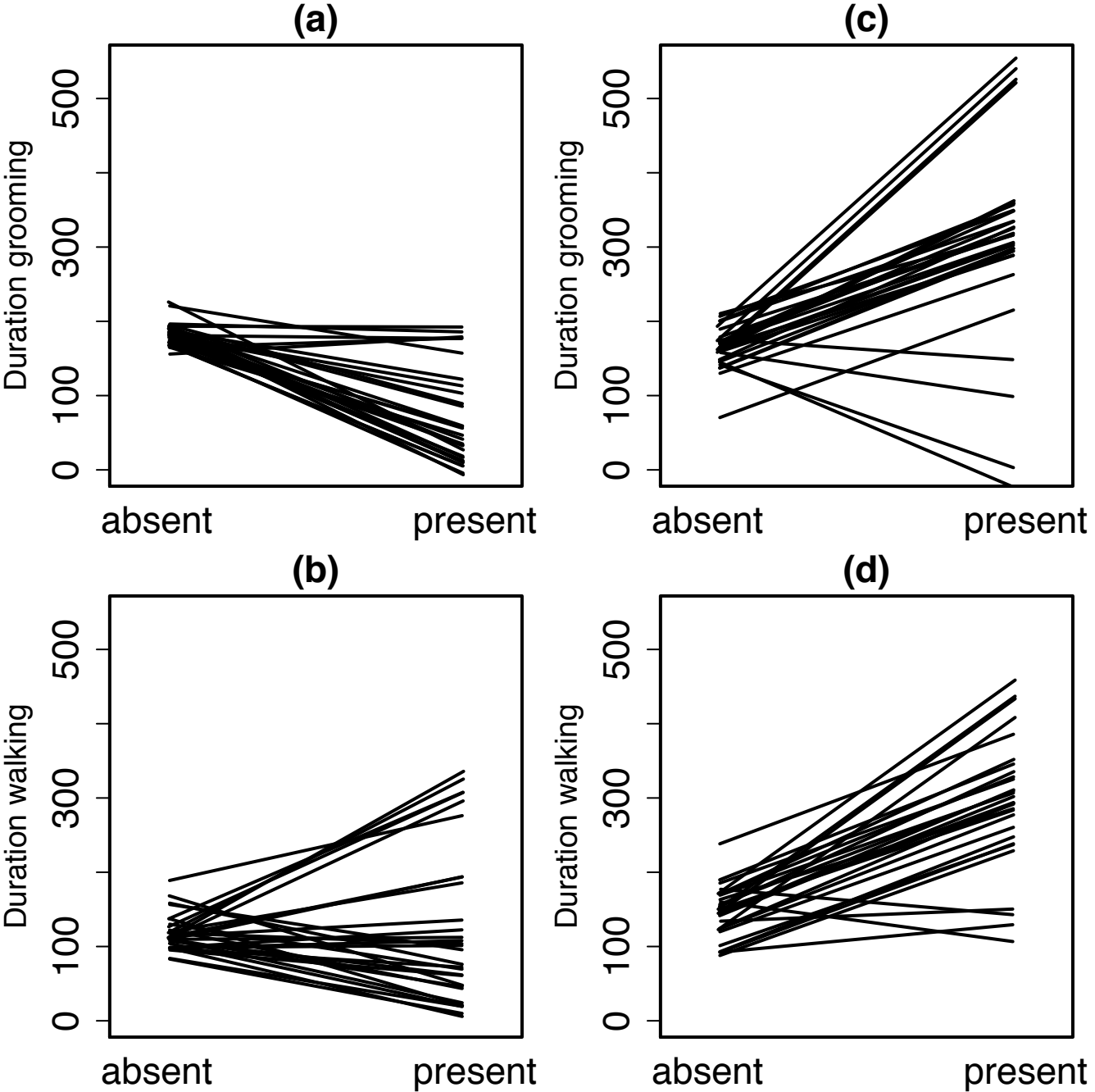

(a)
(b)
(c)
(d)
Duration grooming
Duration walking
0
100
300
500
absent
present

Figure 3

Inter individual behavioural variation in response to predators is present in natural populations. Reaction norms visualize how each individual fruit fly responded to the introduction of a spider (panels a and b) or a mantid (panels c and d) into the assay chamber. Measures are in seconds. Each line corresponds to response of one individual. Estimates are derived from the predicted values for each individual from the mixed models.

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

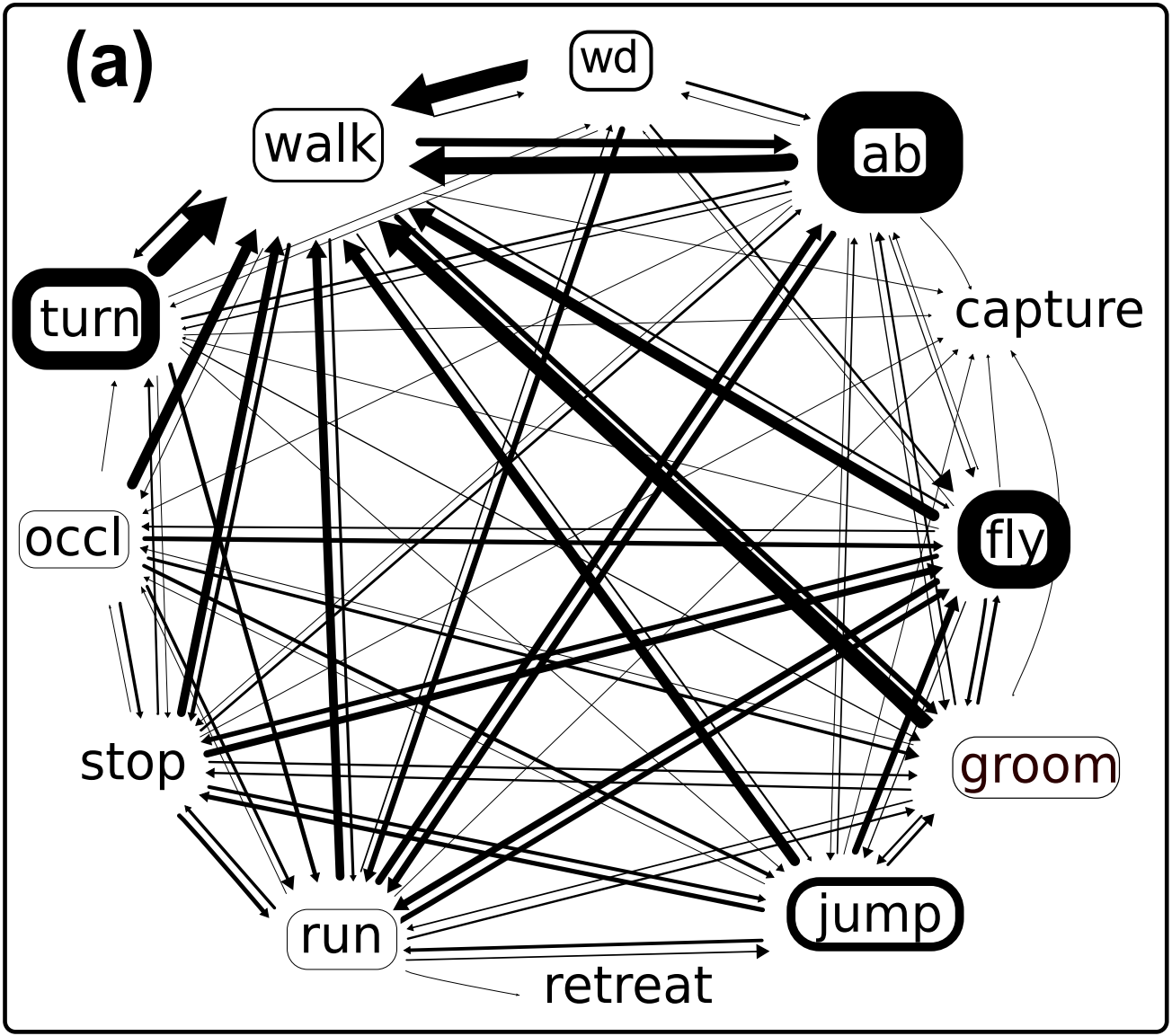
	Intercept	Pred.State	Time
Intercept	122.8 (59.6, 175)	-20.2 (-117, 106)	46.0 (-16.9, 74.5)
Pred.State	-0.05	60.5 (0.13, 100.2)	-18.3 (-47.9, 34.0)
Time	0.8	-0.26	21.5 (2.6, 33.8)

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)

337 lifting). However, while the results of the log-linear analysis (across the whole transition
338 frequency matrix) supported the dependence of current behavioural states on the previous
339 state (resid df=71, deviance=632, $p < 0.001$), the inclusion of predator status did not
340 influence this dependence (resid df = 71, deviance = 59, $p = 0.8$).
341
342 ***Flies perform a previously undescribed retreat behaviour in response to mantids***
343 In contrast to their behaviour in the presence of jumping spiders, the presence of a
344 juvenile mantid had a minimal influence on *D. melanogaster*'s locomotory activity
345 (Figure 2c and 2d, also see ethograms in Figure 1b and Supplement a). Time spent
346 grooming, walking, running and stopping was highly variable (with estimates including
347 zero) in the presence of a juvenile praying mantid (Figure 2d, Supplement b Figures S1
348 and S2). Grooming decreased by 20% (95% CI: 83% decrease to 36% increase),
349 locomotion increased by 9% (13% decrease to 32% increase) and stopping increased by
350 40% (8% decrease to 85% increase). Similarly, the presence of a mantid did not influence
351 the frequency at which *D. melanogaster* tended to perform most instantaneous behaviours
352 (Figure 2c, Supplement b Figure S4). However, as was observed in the presence of
353 spiders, flies substantially increased the frequency of abdominal lifting, from 0.45/minute
354 to 5.6/min (95% CI: 2.56 - 12.17) in the presence of a juvenile mantid (Supplement b
355 Figure S4). Upon encounter with a mantid, half of the individuals (15/30) performed a
356 previously undescribed reversal behaviour (Supplement b video 3), which we term
357 "retreat". As with the zebra spiders, we saw no significant sex specific differences in
358 response to mantids.

359 Although the presence of a mantid had a small effect on fly behaviour, flies did
360 vary considerably in their grooming and walking activities. Indeed, the among-individual
361 variability in proportion of time spent grooming and walking is greater in magnitude in
362 the presence of the mantids than spiders (Figure 3). Evidence for negative co-variation
363 for intra-individual behaviour before and after the addition of the predator was not
364 strongly supported (i.e. 95% CIs for covariances included zero) (Table 2).
365 Transition matrices and transition probability diagrams (Supplement b Figure
366 S7b, Figure 4b and Tables S3, S4, S7 and S8) show patterns of temporal association
367 among behaviours. In response to juvenile mantids, the transitions diagram is less
368 dispersed than that in the presence of jumping spiders (Figure 4), suggesting that the
369 degree of association between behaviours in the presence of mantids is more extreme.
370 While most behaviours (abdominal lift, fly, groom, jump, run, stop, and turn) tend to
371 transition to walking, we also see stronger associations between other pairs of behaviours.
372 For example, after performing the retreat behaviour, flies often either performed the
373 abdominal lift or turn, while flight is often followed by stopping. These observations are
374 supported by the findings that in the absence of mantids, 12 transitions showed
375 significant deviations from expectations (Supplement b, Table S4). In comparison, in the
376 presence of mantids 23 transitions showed a significant deviation from expected values
377 (Supplement b, Table S3). Interestingly, as with the spiders the log-linear model supports
378 the non-independence of behavioural states (resid df=71, deviance=1054, $p < 0.001$), but
379 not for the additional influence of predator state on this non-independence (resid df = 71,
380 deviance = 72, $p=0.4$).
381


(a)
wd
walk
ab
turn
capture
occl
fly
stop
groom
jump
run
retreat

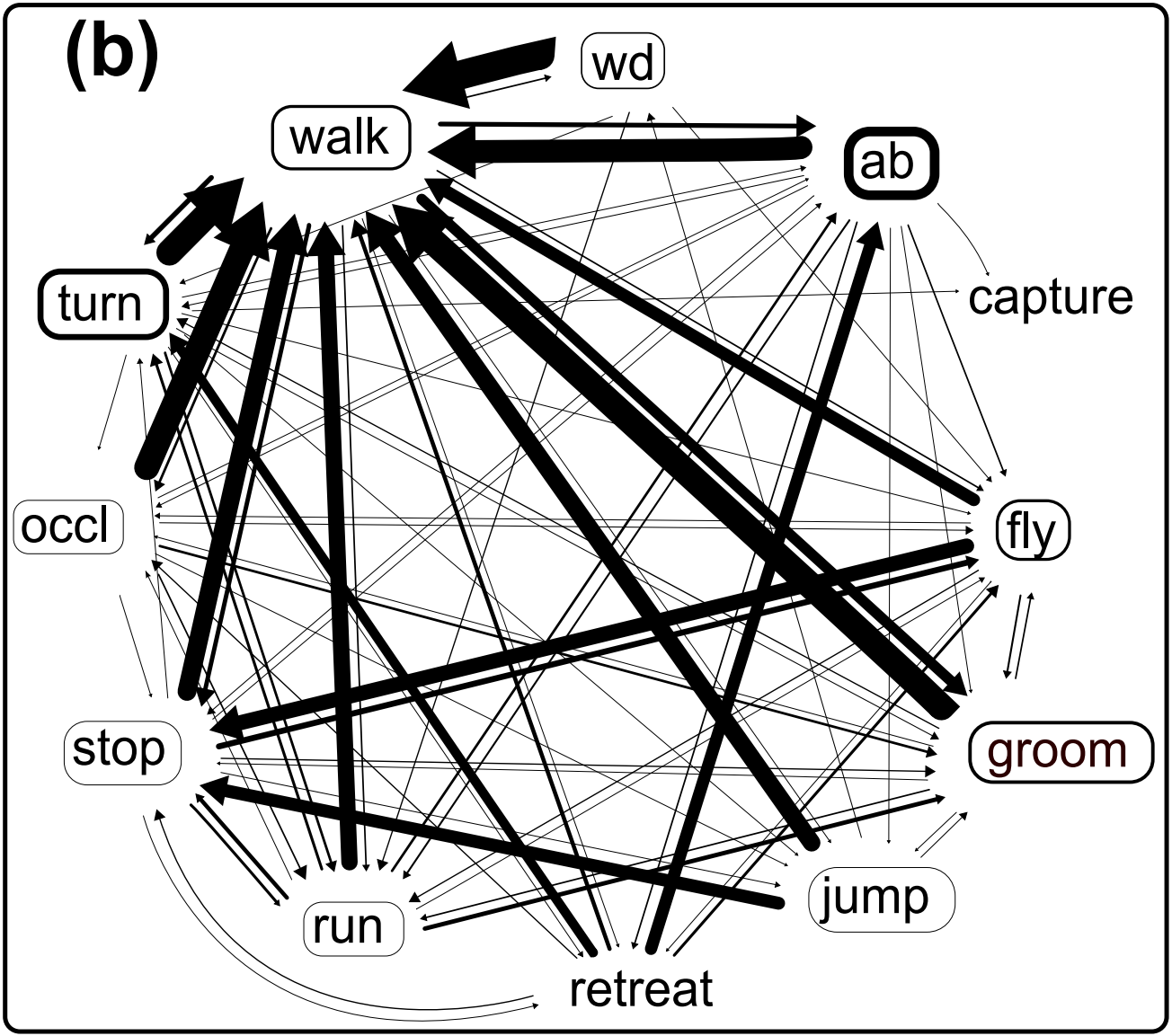

(b)
wd
walk
ab
turn
capture
occl
fly
stop
groom
jump
run
retreat

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 indicates 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.

DISCUSSION

Prey organisms can alter their behaviour to reduce the likelihood of detection, capture or encounter with a predator (Lima, 1998). For example, when predators are present, ground squirrels dedicate more time to vigilance behaviours (like scanning for a predator, see (Bachman, 1993) and some aquatic insects spend more time in refuges (Kohler & McPeek, 1989). These changes in behaviour may alter the use of resources, and potentially the fitness of an organism. However, the nature and intensity of non-consumptive effects of a predator on its prey are a function of several predator specific factors, one of which is the predator's hunting mode (Preisser, Orrock & Schmitz, 2007). Predator hunting mode, i.e., the set of behavioural strategies that a predator employs to pursue and capture its prey (Schoener, 1971; Huey & Pianka, 1981; Schmitz, 2008) can be an important determinant of a prey organism's anti-predatory behavioural response (Schmitz, 2008). In this study, we describe the anti-predatory behavioural repertoire of a natural population of *Drosophila melanogaster* in response to predation by the zebra jumping spider (*Salticus scenicus)* and juvenile Chinese praying mantids (*Tenodera aridifolia sinensis*). Among other characteristics, zebra spiders and praying mantids differ in their hunting mode. While we discuss our findings with respect to hunting mode differences, we recognize that other attributes differing among the predators may contribute to the observed differences in prey behavioural repertoires. However, as our experimental design was meant to minimize the effects of many possible confounding factors (e.g. time of day, temperature, humidity) it seems likely that, in part, our results reflect hunting mode differences.

404 In response to active hunters (those that constantly patrol for prey), we predicted
405 that fruit flies would increase their overall activity levels (including flight) in order to
406 maintain maximum distance from the predator at all times; To reduce the likelihood of an
407 encounter with an ambush predator however (i.e., a predator that only attacks when a
408 prey organism wanders in to its strike zone), we predicted that *D. melanogaster* would
409 respond by decreasing locomotory activities (Schmitz, 2008). Our results, however, were
410 only partially in line with these predictions. While the actively hunting jumping spiders
411 induce a clear increase in overall activity, we found the presence of juvenile mantids- our
412 ambush predators- to have minimal influence on fruit fly activity levels (Figure 2,
413 Supplement b Figure S2). It has been previously argued that ambush predators might be a
414 predictable source of threat to prey organisms (Preisser, Orrock & Schmitz, 2007;
415 Schmitz, 2008) as opposed to the diffuse and variable threat imposed by active hunters
416 (Schmitz, 2008). Therefore, it is perhaps surprising that fruit flies show a stronger
417 behavioural response to the threat of active hunters (zebra jumping spiders). However,
418 our predictions are based on studies on a grasshopper and its two predatory spider species
419 that differ in hunting mode. Given that selection pressures faced by adult diptera are
420 different from those experienced by grasshoppers (orthoptera), such predictions may not
421 be generalizable. Several factors including body size and dispersal patterns may
422 contribute to this difference. Many species of jumping spiders, including *S. scenicus,* are
423 often seen in the natural habitat of *D. melanogaster* (personal observations of A.P., C.P.
424 and I.D.), and are likely to be ecologically relevant predators of *Drosophila*. Mantids
425 however, are rarely found in areas where fruit flies are abundant (personal observations
426 of A.P. and I.D.), at least in Eastern North America. Therefore, it is likely that fruit flies,

427 having experienced a longer evolutionary history with small jumping spiders, are better
428 able to recognize these spiders as a threat. In addition, the disturbance created by a
429 constantly patrolling zebra spider may be partly responsible for the increased activity
430 levels seen in *D. melanogaster* (either due to actual mechanical disturbance or because
431 flies are able to detect moving objects quicker than stationary ones). In this study, we are
432 unable to tease apart the effects of evolutionary recognition versus constant mechanical
433 disturbance on the differences in flies' activity levels. Further experimentation with
434 harmless but constantly moving heterospecifics (such as field crickets) or immobilized
435 active hunters might be useful in addressing these issues.

436 We also identified a number of (to our knowledge) undescribed behaviours of *D.*
437 *melanogaster,* potentially relating to its interactions with predators. The behaviour we
438 called "stopping" (Table 1) was observed numerous times after a direct (but failed) attack
439 by a spider (Supplement 3 video 1). While *D. melanogaster* will spend time without any
440 ambulatory activity (walking, running), they are almost always observed to be active
441 (generally grooming) during these periods. However, when fruit flies performed the
442 stopping behaviour, there was a complete lack of movement on the part of the fly, even
443 when video was viewed at a few frames/second. When a fruit fly was "stopped", the
444 spider had to search for the fly, irrespective of the physical proximity between the spider
445 and the fly. In salticids, while the principal eyes have high spatial acuity, secondary eyes
446 are primarily used to detect moving objects (Harland, Jackson & Macnab, 1999; Land,
447 1971). Because salticids are unable to accommodate by changing the shape of their lens,
448 they need to extensively sample their visual field to see details in object shape and form
449 (Harland, Jackson & Macnab, 1999; Land, 1971; Blest, Hardie & McIntyre, 1981).

450 Scanning for prey by such sampling is likely a slow process unless guided by the motion
451 sensing peripheral eyes, giving motionless prey the advantage of staying hidden (at least
452 for a few seconds) while in plain sight of their salticid predator. Thus, *D. melanogaster*
453 may be using the "stopping" behaviour as a potential mechanism to reduce the likelihood
454 of detection by the spider.

455 Additionally, in the presence of both predators, *D. melanogaster* substantially
456 increase the frequency at which it performed abdominal lifts. To our knowledge,
457 abdominal lifting has not been described in *D. melanogaster* literature before and may be
458 relevant in an anti-predatory context. While studying courtship behaviours in female *D.*
459 *melanogaster*, Lasbleiz, Ferveur & Everaerts (2006) described two behaviours perhaps
460 similar to the abdominal lifting described here: abdominal drumming and abdominal
461 extension. Abdominal drumming (described as "quickly repeated vertical movements of
462 the abdomen which is tapped on the substrate") was only seen in males during courtship
463 display, and abdominal extensions (described as " abdomen raised by 15-30 degrees")
464 were also closely associated with courtship. Because abdominal lifting was often directed
465 at a predator or followed a failed predatory encounter, we suspect abdominal lifting to be
466 different from abdominal extensions and abdominal drumming, and with a possibly anti-
467 predatory function. We speculate that if abdominal lifting is indeed anti-predatory, it
468 could function in one of several possible ways. First, abdominal lifting may be a signal of
469 prey condition directed at the predator as a form of pursuit deterrence, comparable to
470 stotting in the Thomson's gazelle (FitzGibbon & Fanshawe, 1988). Second, because *D.*
471 *melanogaster* are often surrounded by conspecifics, abdominal lifting may be a means
472 though which one fly warns its conspecifics of the presence of a potential threat (similar

473 in function to fin flicking in tetras, (Brown, Godin & Pedersen, 1999). Finally it may be
474 an indication of some sort of physiological priming of the fly in preparation for a fight-
475 or-flight response. Determining whether it is a specific anti-predator behaviour, as well as
476 the details of its function need to be a focus of future work.

477 In response to the juvenile praying mantids, half of the fruit flies we observed
478 (15/30) performed a reverse walking behaviour which we have called "retreat", where the
479 flies walked in reverse, away from the predator (supplement b, video 3). This was often
480 (but not always) interspersed with the abdominal lifting behaviour. Phenomenologically,
481 this behaviour may be similar to that described in Bidaye et al. (2014). Bidaye el al.
482 (2014) identified neurons that upon activation changed walking direction in *D.*
483 *melanogaster*. Bidaye et al's reverse walking behaviour appears to be a smooth and
484 continuous behaviour, whereas "retreat" was often discontinuous and accompanied by
485 abdominal lifting. If the two "retreat" behaviours are related, the observed disassociation
486 between retreat and abdominal lifting as well as its continuous nature (in Bidaye el al.,
487 2014) may be a function of how the neurons were perturbed.

488 We also investigated how the presence of the different predators may influence
489 non-random associations among behaviours. We observed that in the presence of both
490 predators there was an increase in the number of behavioural transitions that deviated
491 from expectations under independence (from 12 to 23 with the mantid, and 8 to 13 with
492 the spider). Despite this, the log-linear model (analysing the whole transition frequency
493 matrix) did not support the influence of predator state on the frequencies of transitions.
494 This may be partly due to the relatively modest sample sizes (in terms of both individuals

495 and transitions among behaviours). Further work is necessary to validate and extend this
496 sequential analysis.
497 While we show that there are some predator hunting-mode specific behavioural
498 differences in *D. melanogaster's* anti-predator response, we reiterate two important
499 caveats. First, although the primary distinction between the zebra jumping spider and
500 juvenile Chinese praying mantids as predators is their hunting-mode, other factors
501 between these species (for example, size, colour, odour) may also influence differences in
502 fruit fly behaviours. Replicating the observations with other predator pairs that differ in
503 hunting-mode is necessary to confirm hunting-mode's influence on anti-predatory
504 repertoires. Secondly, our assay chambers are an artificial environment and do not
505 resemble the conditions under which *D. melanogaster* face predators in the wild. Due to
506 the nature of our assay chamber, *D. melanogaster* were unable to employ behavioural
507 strategies that may reduce encounters with predators (e.g., utilizing a refuge). Therefore
508 we were only able to describe the capture-deterrence repertoire of *D. melanogaster*
509 behaviour. We believe that our study is a necessary first step to describing and
510 documenting the complete anti-predatory behavioural repertoire of *D. melanogaster* and
511 we foresee future work to be conducted in a modified chamber, under more "natural"
512 conditions. Doing so will allow us to take this premier model genetic system and make it
513 into an ecological model as well.
514

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Supplemental material

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Supporting information

Differential response to spiders versus mantids

Because spider and mantid population densities vary by season, we had to temporally segregate the spider assays from the mantid assays. We conducted all spider observations between October and December 2012 and all the mantid observations between March and May 2013. Comparing time allocation and frequencies of occurrences in the predator absent state between the two predator treatments suggest that behavioural modifications were predator induced, and not due to seasonal effects (Figure S5 and S6). Although the assays were carried out under highly controlled conditions, to confirm that predator species-specific behavioural differences were not confounded with seasonal differences in behaviour, we performed 6 additional assays (alternating between spider and mantid treatments) within the span of one week. The control experiments show no evidence of confounding effects of season with *D. melanogaster*'s anti-predator behavioural repertoire (Table S9, S11 and S12 below). Ethograms are shown in Supplement a. Furthermore, to confirm that the disturbance we caused (to the assay

chamber) during the addition of a predator did not confound behavioural responses to the predator, we did 3 “no predator” control assays. For these “no predator” controls, instead of adding a predator to the arena, we caused a mild disturbance (~ to intensity of disturbance caused while adding the predator) without actually adding any predator. We found that disturbance caused during predator addition was not responsible for observed behavioural modifications (Table S10 and S13). Finally, “no predator” controls also ruled our temporal differences in fruit fly activity levels (Table S10 and S13)

Supplemental figures

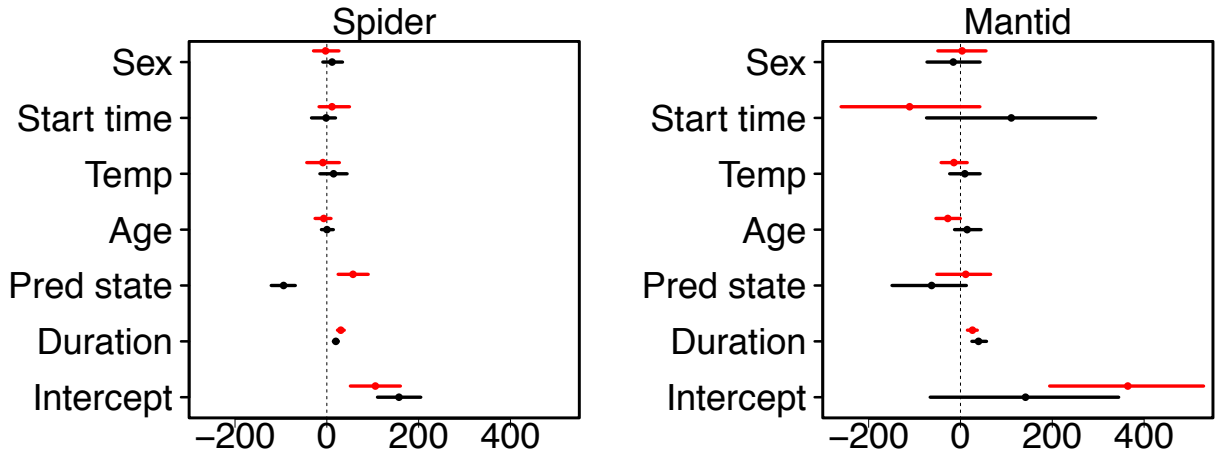

Figure S1

Presence of jumping spiders causes fruit flies to walk more and groom less, whereas the presence of mantids causes weaker, more variable (and not significant) changes in fruit fly activity levels. Here we show coefficient plots from the output of mixed effects models using the package MCMCglmm to visualize duration of two behaviours (**grooming in black** and **locomotion in red**) as a function of predator state (present vs absent of spiders, left panels and mantids, right panels), time spent in the assay [Time (cent.)] total, sex of the fly, start time of the assay, temperature in the room and age of the fly. The continuous covariate “Time” was centered

around the mean and therefore reflects the average increase in time spent per minute of the assay. Estimates are in seconds. Error bars are ± 95% CI.

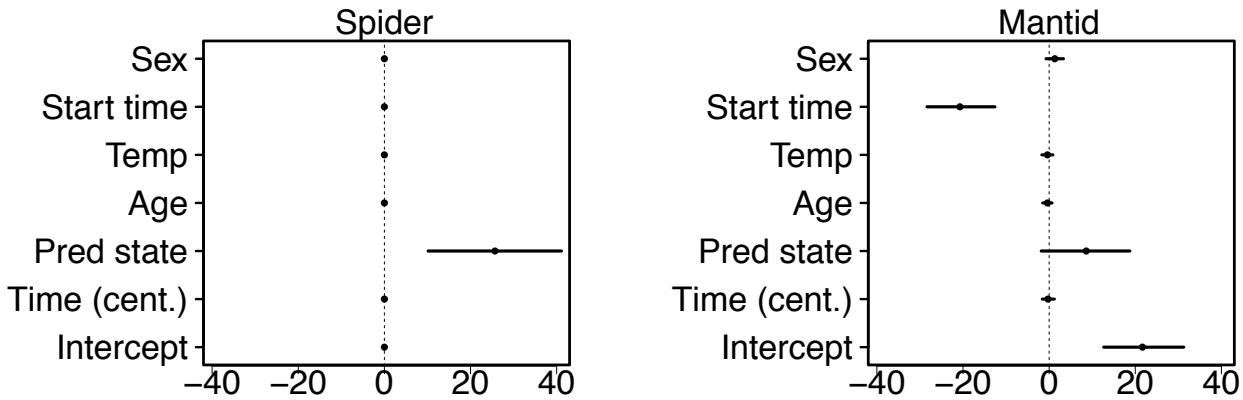

Figure S2

Fruit flies "stop" significantly longer in the presence of spiders (left panel), and to a much lesser extent (and not significantly) in the presence of mantids (right panel). Here we have coefficient plots made from the output of mixed effects models using the package MCMCglmm to visualize duration of "stopping" as a function of predator state (present vs absent), time spent in the assay [Time (cent.)], sex of the fly, start time of the assay, temperature in the room and age of the fly. Estimates are in seconds. Error bars are ± 95% CI. The continuous covariate "Time" was centered around the mean and therefore reflects the average increase in time spent per minute of the assay. Although assays were performed between 9 am and 12 pm each day, start time for the mantid assays significantly affected the total time that flies spent "stopping".

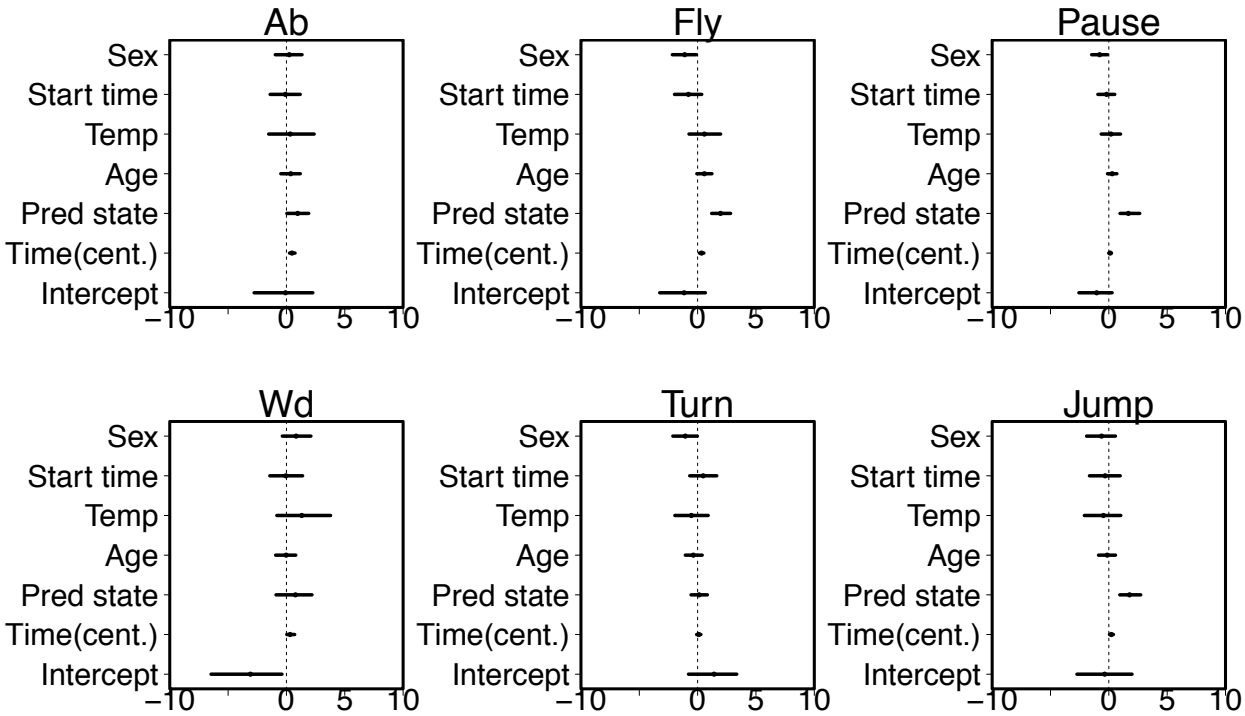

Figure S3

In the presence of spiders, fruit flies increased the frequency with which they performed flights, pauses and jumps. Here we show coefficient plots made from the output of mixed effects models where the events were modeled using a poisson generalized linear mixed model with a log-link function fit using the `MCMCglmm` function, and estimates remain on a natural log scale. Coefficient plots were used to visualize frequency of each individual behavioural event (ab, fly, pause, wd, turn and jump) as a function of predator state (present vs absent of a spider), time spent in the assay [Time (cent.)], sex of the fly, start time of the assay, temperature in the room and age of the fly. All estimates are scaled to number of events per minute. Error bars are ± 95% CI.

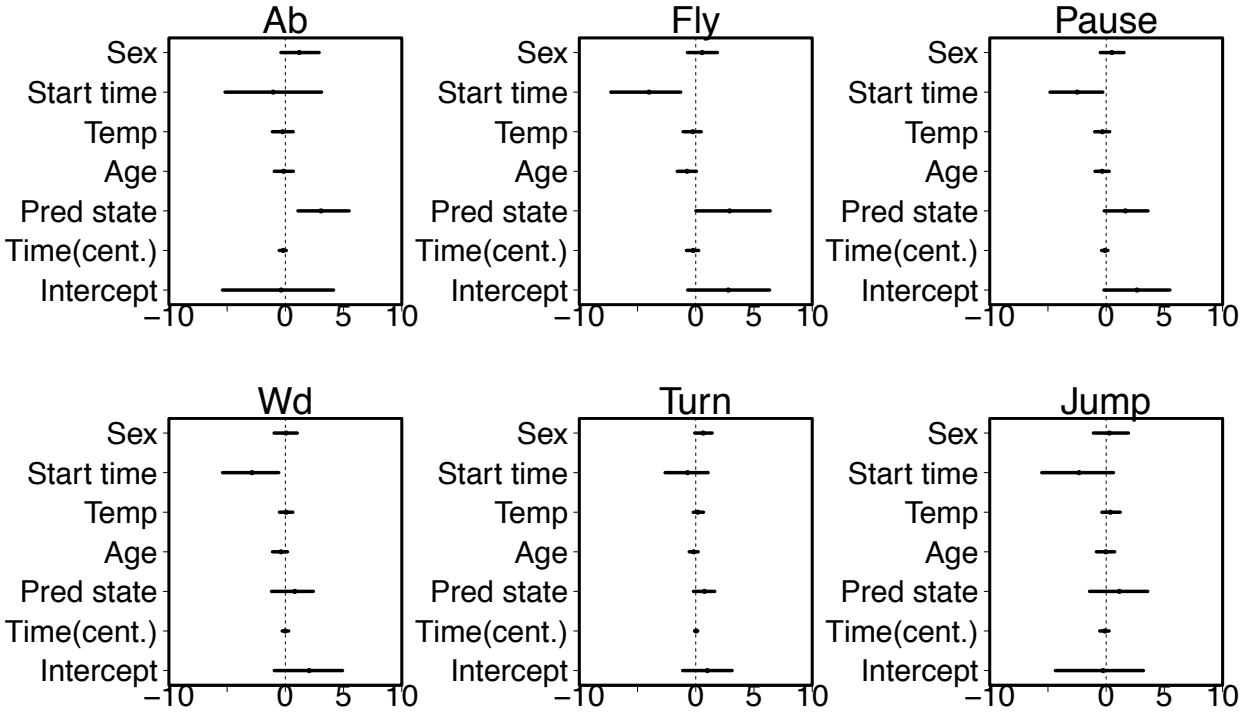

Figure S4

Fruit flies performed abdominal lifts are a higher frequency in the presence of a juvenile mantid. Coefficient plots made from the output of mixed effects models where the events were modeled using a poisson generalized linear mixed model with a log-link function fit using the `MCMCglmm` function, and estimates remain on a natural log scale. Coefficient of the model output are used to visualize frequency of each individual behavioural event (ab, fly, pause, wd, turn and jump) as a function of predator state (present vs absent of a mantid), time spent in the assay [Time (cent.)], sex of the fly, start time of the assay, temperature in the room and age of the fly. All estimates are scaled to number of events per minute. Error bars are ± 95% CI. Although assays were performed between 9 am and 12 pm each day, start time for the mantid assays significantly affected the frequency at which *D. melanogaster* performed the “Fly”, “Wd” and “Jump” behaviours.

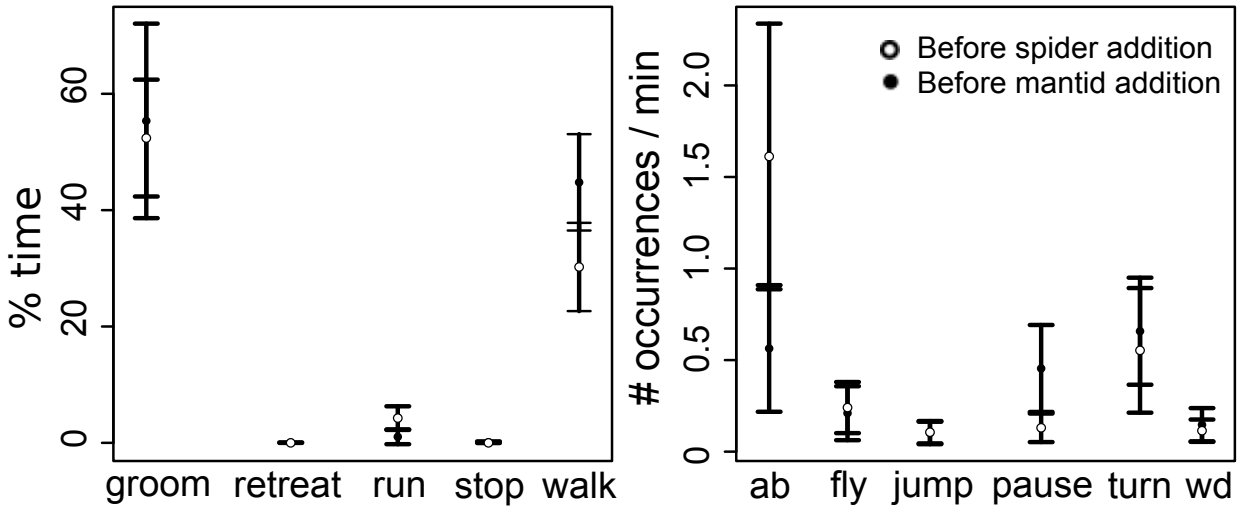

Figure S5

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.

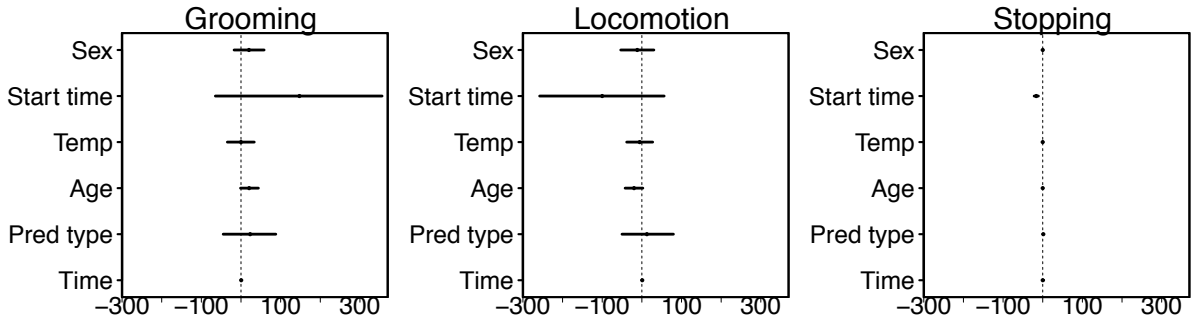

Figure S6

Seasonal differnces in fruit fly behaviours did not confound behavioural differences induced by different hunting-modes. Flies measured before the addition of a spider did not differ in behaviour from flies measured before the addition of a mantid.

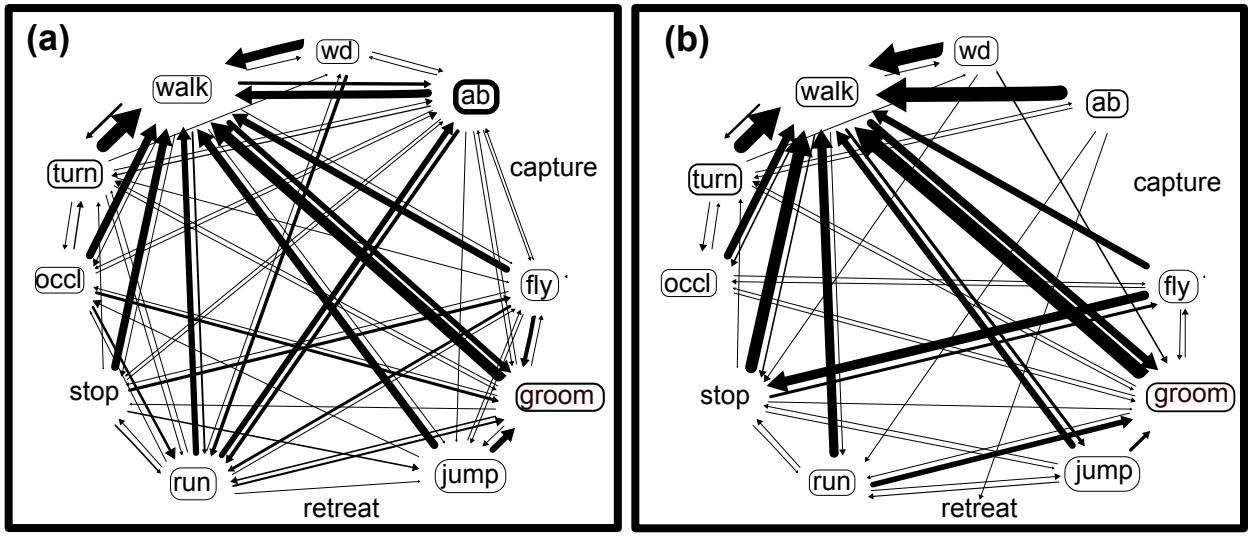

Figure S7

a) A diagram representing probability of transitioning from one fly behaviour to the other when individuals were measured before the addition of a spider **b)** A diagram representing probability of transitioning from one fly behaviour to the other for individuals measured before the addition of a juvenile mantid. Thickness of arrows indicates 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.

Supplemental tables

Table S1 Transition frequency matrix when a spider was present in the chamber. Each row represents the number of times one behaviour (row name) transitioned to another behaviour (column name). Numbers in blue represent transitions that occurred more often that expected under a model of independence, whereas numbers in red are transitions that occurred less often 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

Table S2 Transition frequency matrix before a spider was added to the chamber. Each row represents the number of times one behaviour (row name) transitioned to another behaviour (column name). Numbers in blue represent transitions that occurred more often that expected under a model of independence, whereas numbers in red are transitions that occurred less often than expected (see methods)s.

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

Table S3 Transition frequency in the presence of a juvenile praying mantid. Each row represents the number of times one behaviour (row name) transitioned to another behaviour (column name). Numbers in blue represent transitions that occurred more often that expected whereas numbers in 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

Table S4 Transition frequency matrix before a juvenile mantid was added to the chamber. Each row represents the number of times one behaviour (row name) transitioned to another behaviour

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

Table S5 Transition probability from one behaviour (row name) to the other (column name) in the presence of a zebra jumping spider. 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).

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

Table S6 Transition probability from one behaviour (row name) to the other (column name) before a zebra jumping spider 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 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

Table S7 Transition probability from one behaviour (row name) to the other (column name) in the presence of a juvenile praying mantid. 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).

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.

		Grooming		**Walking**		**Running**		**Stopping**		**Retreat**	
Treatment	**Individual**	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.

		Pause		Turn		Ab		Wd		Fly		Jump	
Treatment	**Individual**	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

Table S12 Coefficients from a linear model (lm) for control individuals meansured before and after the addition of a mantid. Estimate of posterior means are similar to those of the main mantid 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

Table S13 Coefficients from a linear model (lm) for control individuals meansured before and after a disturbance. Despite low sample sizes, it is clear that disturbance had minimal effect on fruit fly behaviours.

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

Links to videos describing novel behaviours

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

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

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