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 or 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 the predator's hunting mode while describing differences in 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 them 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. 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[1-7]. Predator hunting-modes, i.e., the set of behaviours that predators employ to pursue and capture their prey [8-10], have been shown to induce distinct prey responses [10,11] that in turn influence the productivity of ecological communities. For example it has been found that habitats dominated by active hunters had lower species evenness and higher above-ground net primary productivity compared to habitats dominated by ambush hunters [10]. The authors suggest the observed differences in prey productivity to be driven by hunting mode specific trade-offs between foraging and seeking refuge [10]. However, studies describing the effects of predators on prey traits [12-14] have often ignored the role of predator hunting-mode.

In this study we investigate segregating differences in the anti-predatory behavioural repertoire of the model fruit fly, *Drosophila melanogaster*, in response to two predator species differing in hunting modes. Based on [11], 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 ecology and behaviour, including habitat, food resources, and natural enemies (but see [18-24]). Meanwhile, though anti-predator behaviours in general are targets of selection in prey [2,15-17], the genetic bases of such behaviours have seldom been investigated due to lack of molecular genetic tools. Given the range of genetic and genomic tools available for *D. melanogaster,* along with its complex behavioural repertoire and suitability for experimental evolution, understanding

the anti-predatory behaviours persisting in a natural population of the fruit fly brings us one step closer to deciphering the molecular mechanisms of evolutionary response to predation as a selective agent.

Previous work has examined the effects of natural enemies on population and community structures of *Drosophila spp*. For example, Worthen et al [25] studied the effects of predation by staphylinid coleopterans on the coexistence of three *Drosophila* species, and Escalante et al [26] showed that ant predators regulate population densities of wild *D. starmeri* (cactophillic fruit fly). Additionally, in *D. melanogaster per se*, the role of parasites in influencing larval as well as adult behaviour has been extensively studied [17,27-29]. Despite this literature, we know little about the predators of *D. melanogaster* adults in the wild, nor the nature of anti-predatory behaviours segregating in natural populations.

We documented the influence of two predators, the zebra jumping spider (*Salticus scenicus*) and juvenile Chinese praying mantids (*Tenodera aridifolia sinensis*) on the capture-deterrence behaviours of *D. melanogaster* individuals derived from a wild-caught population. The zebra spider is an active hunter; locating prey visually (with an extensive visual field attained by antero-medially positioned simple eyes) [30,31]. Where as mantids are generally ambush predators; waiting for prey to enter their attack range [32]. Despite numerous differences, Zebra spiders and juvenile Chinese mantids are similar in two relevant ways. First, both species primarily detect prey visually [33-36] and are likely incapable of depth perception when their prey item is motionless [36,37]. Second, small adult diptera account form a substantial proportion of the diet of both predators in the wild [38,39].

Based on the findings of Schmitz [11], 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 substantially 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. We discuss our results within the broad context of conditionally expressed behaviours as they relate to predator hunting mode, as well as specifically with respect to broadening our understanding of the behavioural ecology of *D. melanogaster*.

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^{0}C (+/- 1^{0}C), 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^{0}C 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

using socially naïve flies in our assays, we were able to establish a consistent baseline of social experience among all individuals, allowing us to eliminate the potentially confounding influence of variation in social experience on behaviour that is well-documented in *Drosophila* [40-44].

Spiders

S. scenicus individuals were collected throughout the spring/summer of 2012 on the campus of Michigan State University. Spiders were housed individually in vials in a room maintained at 23^0C (+/- 1^0C) and 30-50% RH and fed ~5 *D. melanogaster* a week. Prior to use in behavioural assays, spiders were starved for at least 48 hours. Each spider was used in only a single behavioural assay.

Mantids

Mantid egg cases were both collected near the campus of Michigan State University as well as ordered from Nature's Control (Medford, Oregon). Mantid egg 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 [45], 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 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:

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

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)

$\beta_0 \sim N(0, \sigma^2_j)$ 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.

To test for non-random associations in the temporal structure of behavioural patterns we constructed transition frequencies using the "msm" library (version 1.2) [47] 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 with the transition frequency matrices [48] using glm in **R**. As advocated by [48,49] we fit a saturated log-linear model (with lag0, lag1 and PredState as the effects in the model) and tested the influence of deleting the terms (i.e. third order interaction of main terms) on change in deviance. We used modified "Z-scores", adjusted using sequential Bonferroni to assess the deviation of particular cells in the transition frequency matrix from expected values (assuming independence). For the visual transition probability matrices, we combined the behavioural event "pause" with the behavioural state "stop" because 1) we wanted to reduce the complexity of the matrix and 2) the main difference between the two behaviours is that pause is instantaneous and stop has duration. All transition diagrams were constructed in Inkscape [50](version 0.48.2).

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)

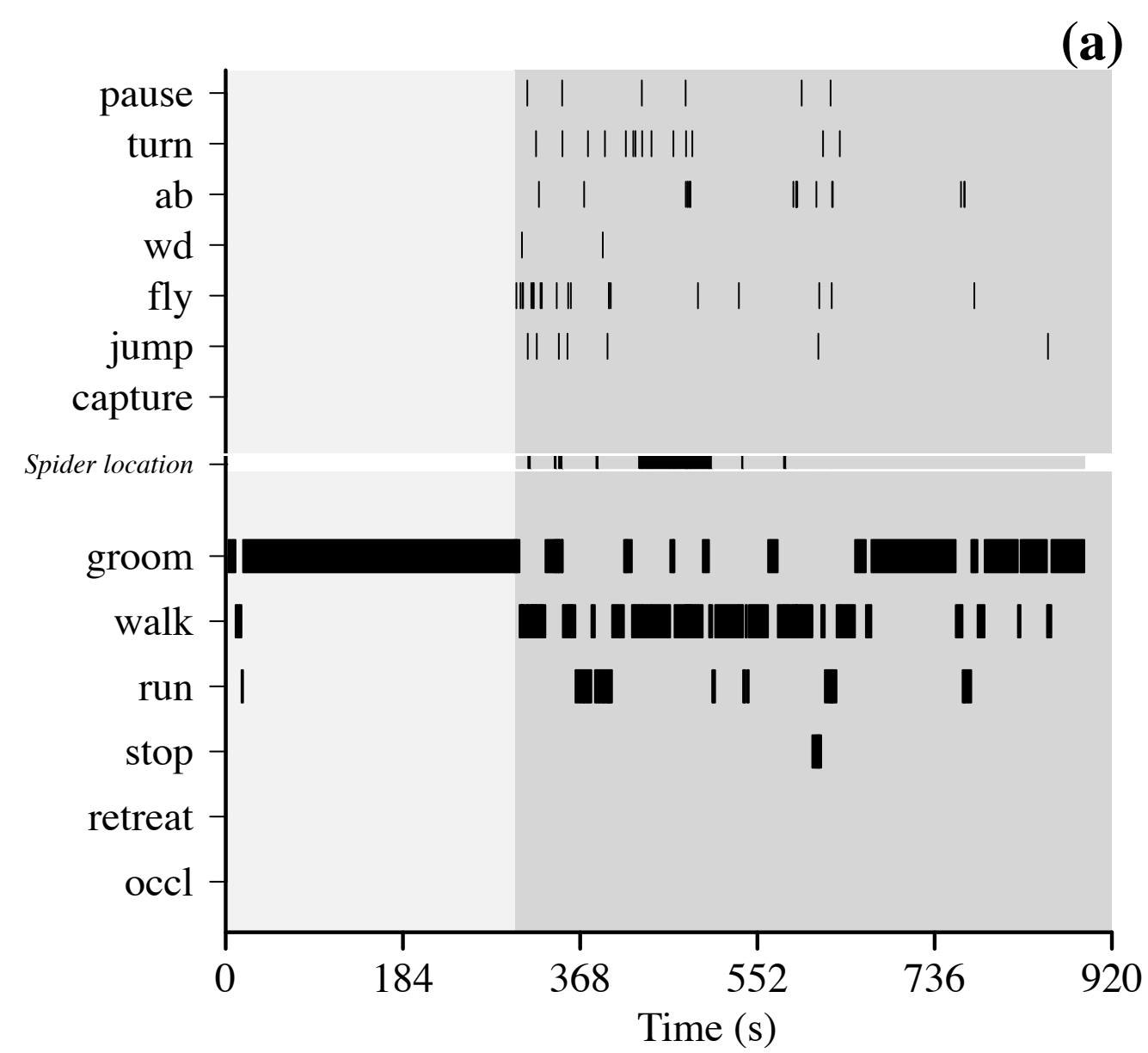
RESULTS

From pilot observations (not included in analysis), we (I.D., A.P. and C.P.) catalogued and described *Drosophila melanogaster* behaviours observed in the presence of a predator (Table 1). Among the behaviours listed in Table 1, abdominal lifting (ab, supplement b, video 1) and retreat (supplement b, video 3), to our knowledge, have not been previously described in *D. melanogaster* literature.

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

To visualize each individual fruit fly's response to the presence of a zebra jumping spider, we generated ethograms (see Figure 1a and Supplement a). For the two predator states (spider present and spider absent) we measured the mean proportion of time dedicated to each behavioural state, as well as the number of occurrences per minute for each behavioural event. When a spider was present, *D. melanogaster* increased the proportion of time it spent walking and running while grooming less (Figure 2 and Supplement b Figure S1). While they were observed at low frequencies prior to the addition of spiders, *D. melanogaster* substantially increased the frequency of pauses, abdominal lifts, jumps and flights (per minute) in the presence of spiders (Figure 2, Supplement b Figure S3). Time allocated to "stopping"; a motionless state that likely aids in capture deterrence (see Videos 2, Supplement c) also increased significantly in the presence of spiders (Supplement b Figure S2). However, when interacting with spiders, flies were only observed to perform the "retreat" behaviour once (of 30 individuals).

Given the design of our experiment, we were able to model the degree to which individuals varied in their responses to the jumping spiders. Individuals varied greatly


(a)
pause
turn
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Spider location
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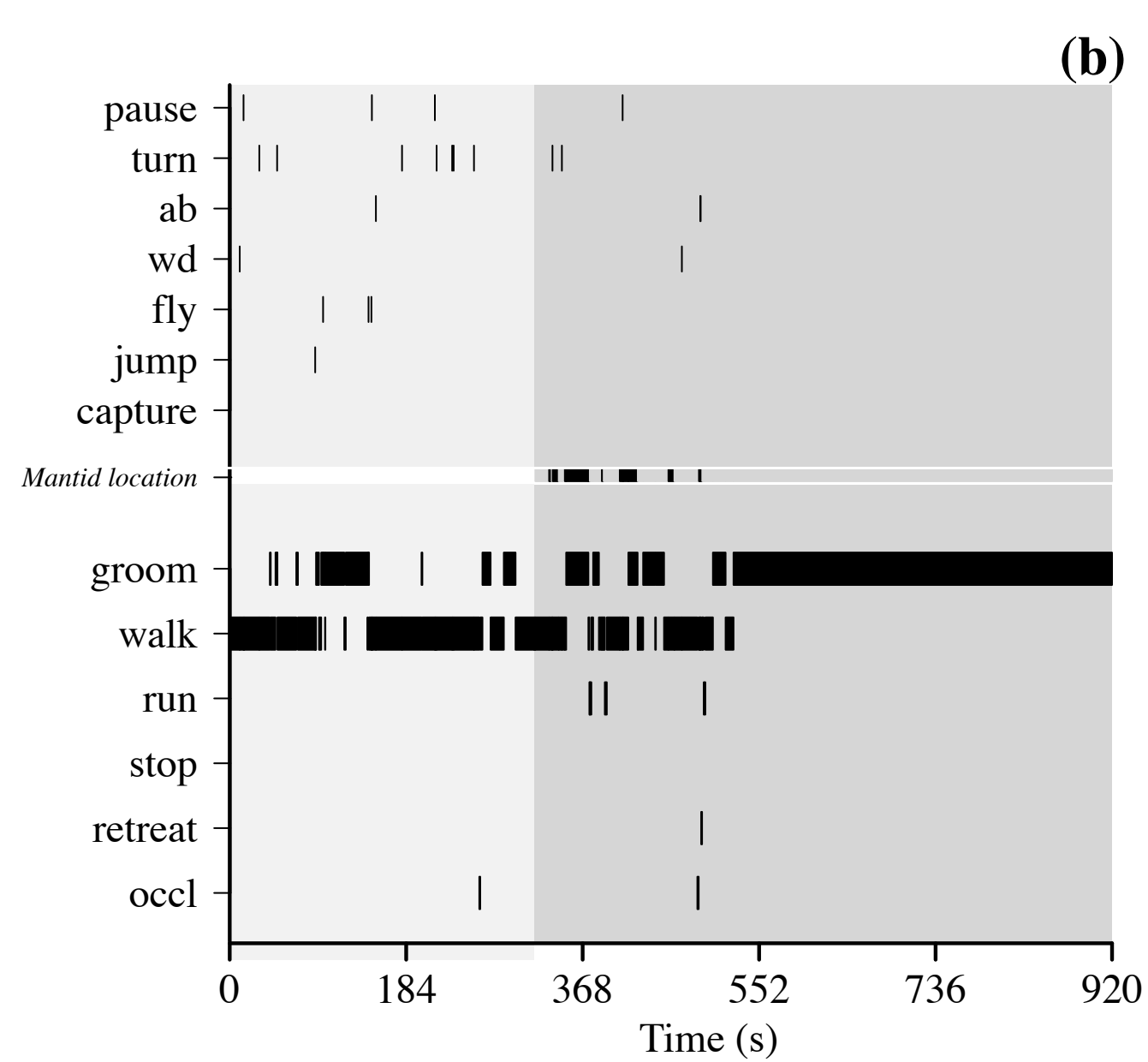
736
920
Time (s)


(b)
pause
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Mantid location
groom
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retreat
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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.

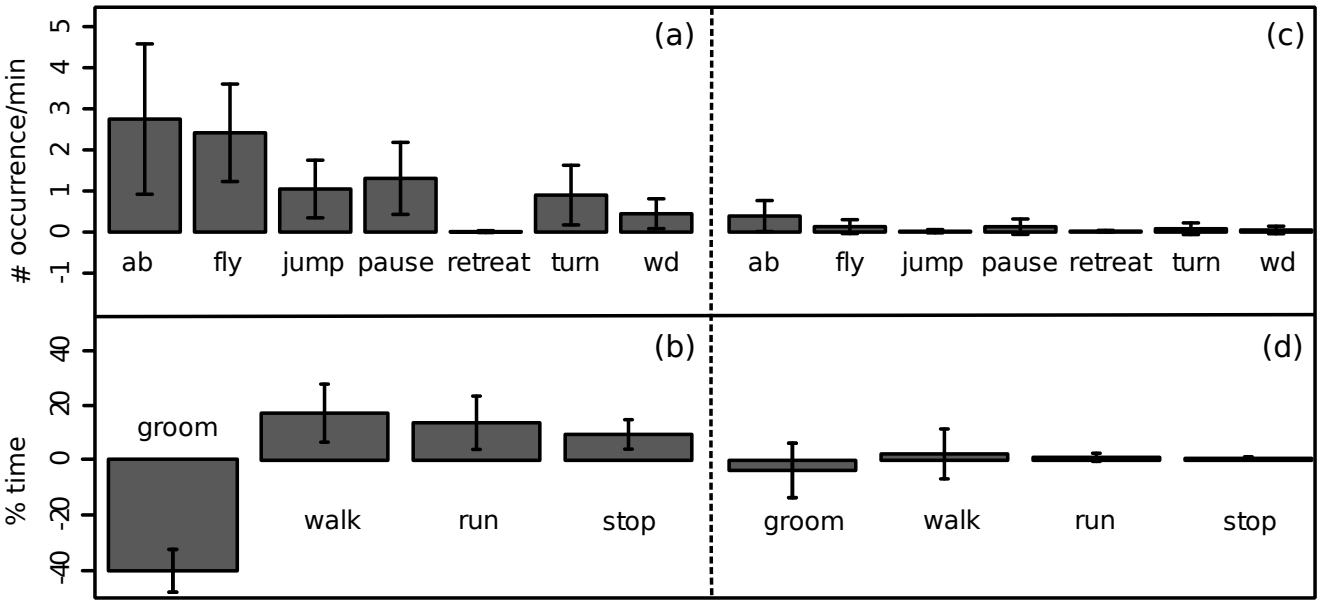

(a)
(c)
(b)
(d)
occurrence/min
% time
5
4
3
2
1
0
-1
40
20
0
-20
-40
ab
fly
jump
pause
retreat
turn
wd
groom
walk
run
stop

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.

both in their baseline activity levels as well as in their propensities to respond to jumping spiders. While most individuals reduced their grooming activity in the presence of predators, the degree to which they did so varied substantially (Figure 3a and 3b). Interestingly, we did not see significant sex specific differences in either frequencies of occurrence (Supplement b Figure S3) or proportion of time allocated (Supplement b Figure S1) to the majority of measured behaviours (But see S3 panels "pause" and "turn"). There was an overall negative correlation between the amount of time individuals spent grooming before and after the addition of the spiders (Table 2). That is, on average, individuals who were more active prior to the addition of the spider reduced their activity to a greater extent in the presence of the spider.

To visualise the temporal associations among behavioural sequences, we constructed transition matrices (Supplement b Tables S1, S2, S5 and S6) and transition probability diagrams for all pairs of behaviours in the absence (Supplement b Figure S7) and presence (Figure 4a) of predators. In response to jumping spiders, transitions among behaviours are somewhat more dispersed (with many connections between behaviours), suggesting that there is weak temporal association between fruit fly behaviours. Indeed these qualitative conclusions are supported based on the Z-scores. In the absence of spiders 8 possible transitions were significant (after controlling for multiple comparison, Supplement b Table S1), while 13 transitions were significant in the presence of the spider (Supplement b Table S2). Most of these differences were due to the increase in behaviours potentially involved with anti-predation activity (i.e. flight, abdominal lifting). However, while the results of the log-linear analysis (across the whole transition frequency matrix) supported the dependence of current behavioural states on the previous

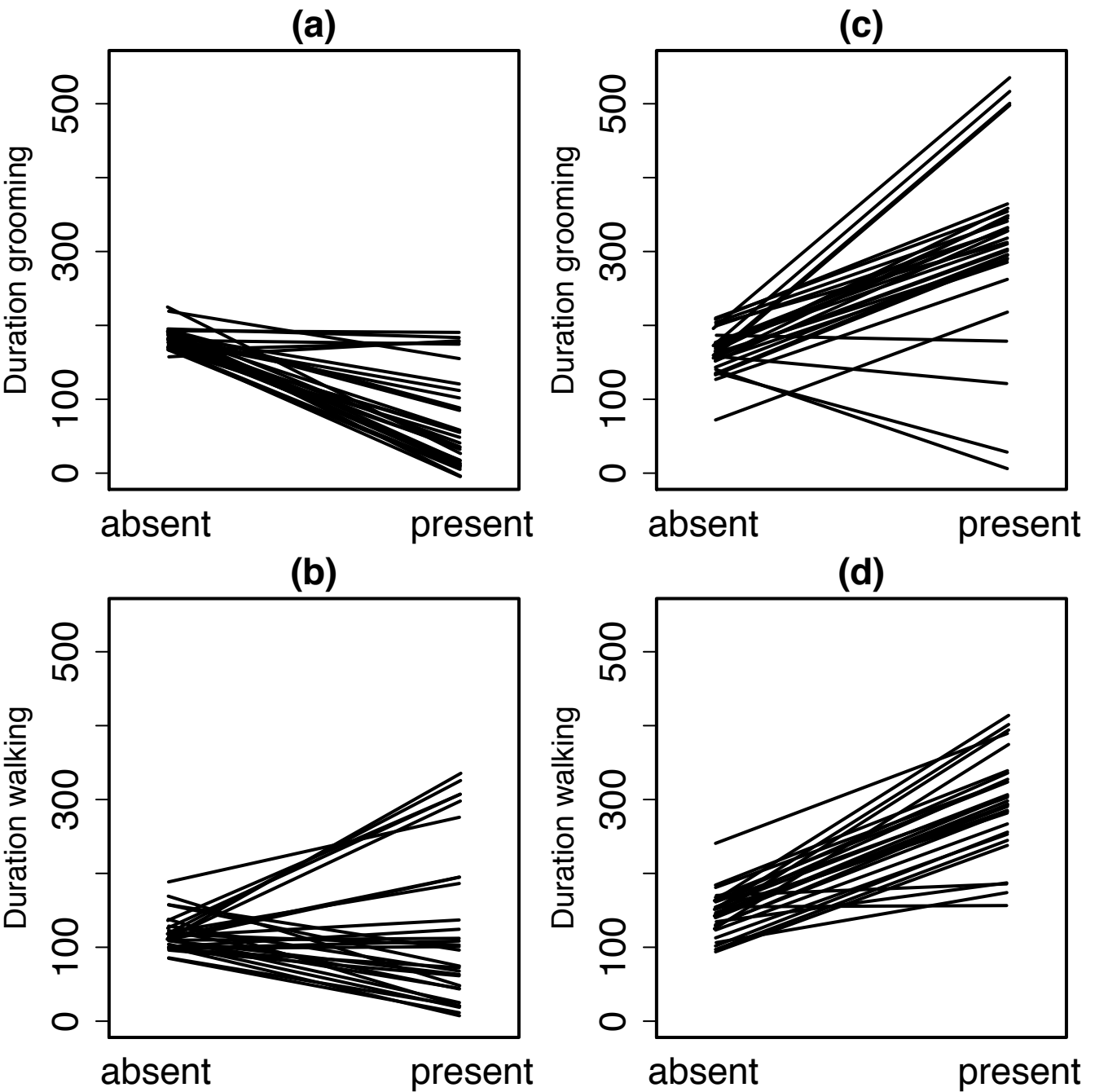

(a)
(b)
(c)
(d)
Duration grooming
Duration walking
0
100
300
500
absent
present

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

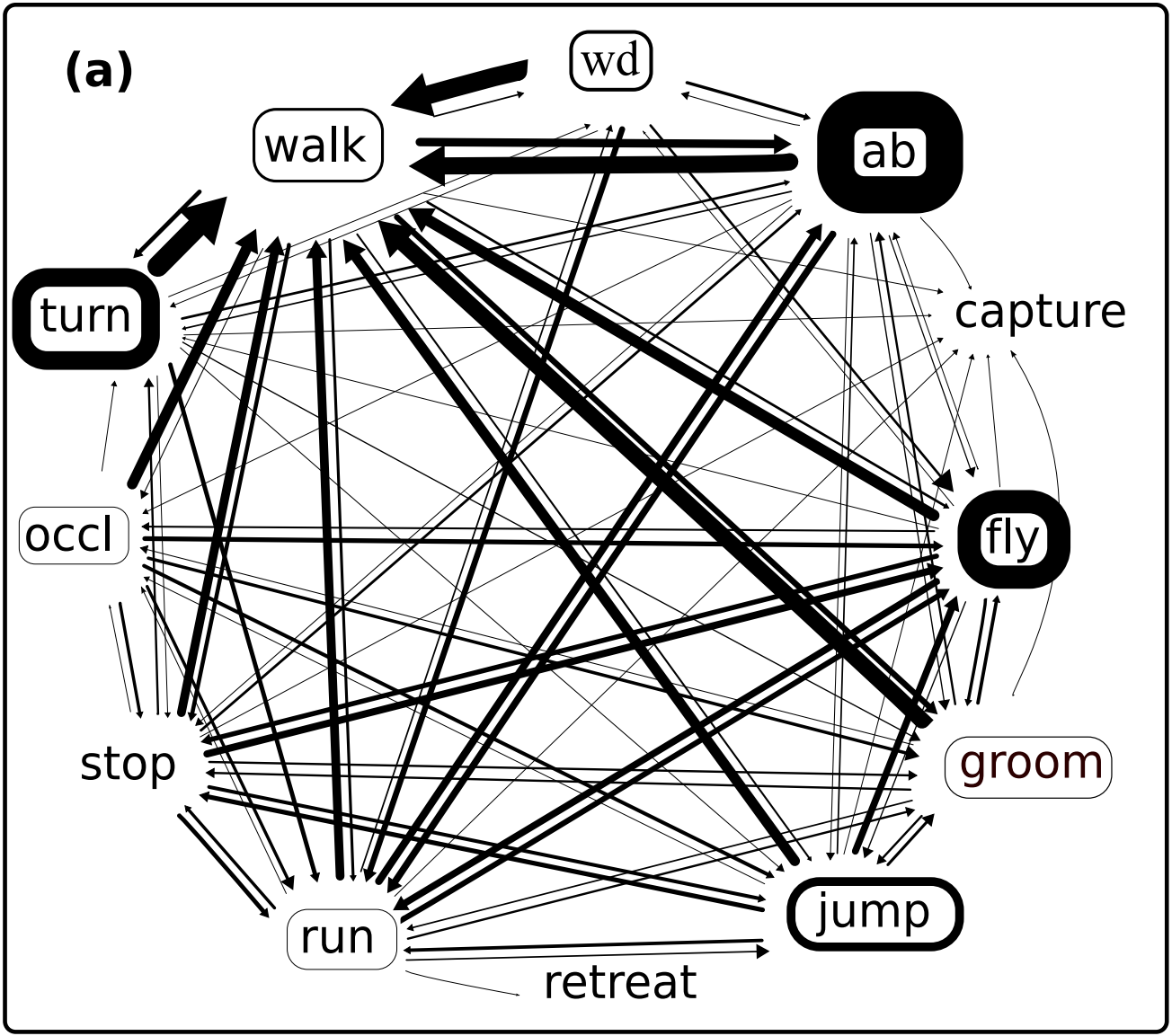
Inter individual behavioural variation in respone to predators is present in natural populations. Reaction norms visualize how each individual fruit fly responded to the introduction of a spider (plannels a and b) or a mantid (pannels c and d) into the assay chamber. Measures are in seconds. Each line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

state (resid df=71, deviance=632, $p < 0.001$), the inclusion of predator status did not influence this dependence (resid df = 71, deviance = 59, $p = 0.8$).

Flies perform a previously undescribed retreat behaviour in response to mantids

In contrast to their behaviour in the presence of jumping spiders, the presence of a juvenile praying mantid had minimal influence on *D. melanogaster*'s locomotory activity (Figure 1, Supplement a). Time spent grooming, walking, running and stopping was largely unaffected by the presence of a juvenile praying mantid (Figure 2d, Supplement b Figures S1 and S2). Similarly, the presence of a mantid did not influence the frequency at which *D. melanogaster* tended to perform most instantaneous behaviours (Figure 2a, Supplement b Figure S4). However, as was observed with spiders, flies performed the abdominal lifting behaviours (ab) at a significantly higher rate in the presence of a juvenile praying mantid (Supplement b Figure S4). In addition, upon encounter with a mantid, half of the individuals (15/30) performed a previously undescribed reversal behaviour (Supplement c video 3), which we term "retreat". As with the zebra spiders, we saw no significant sex specific differences in response to mantids.

Although the presence of a mantid had a small effect on fly behaviour, flies did vary considerably in their grooming and walking activities. Indeed, the among-individual variability in proportion of time spent grooming and walking is greater in magnitude in the presence of the mantids than spiders (Figure 3). The results from the mixed model did not demonstrate as strong support for the negative co-variation between an individual's activity (walking, grooming) before and after the addition of the mantid (Table 2).


(a)
wd
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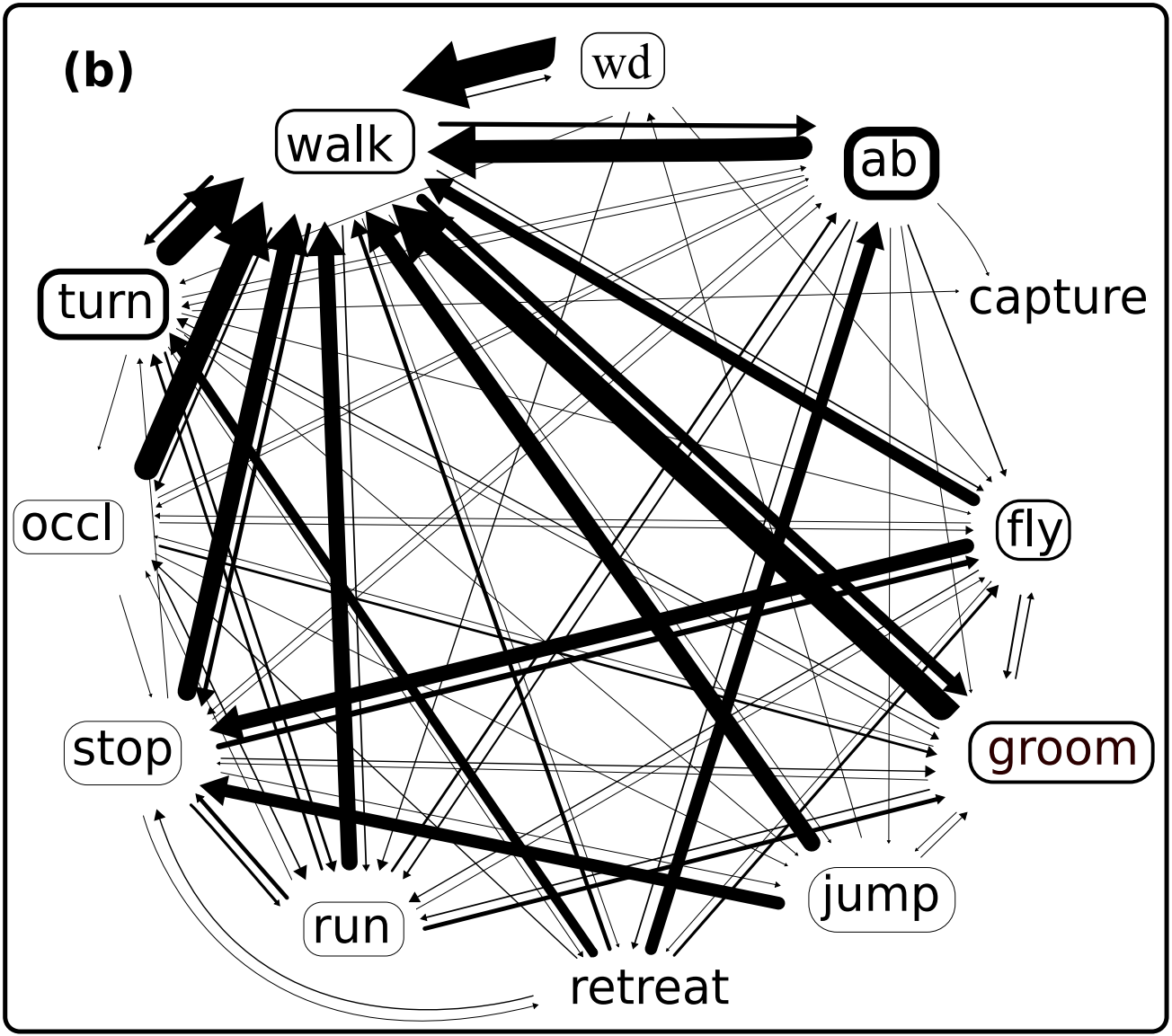
(b)
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fly
stop
groom
jump
run
retreat

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 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.

Transition matrices and transition probability diagrams (Supplement b Figure S7, Figure 4b and Tables S3, S4, S7 and S8) show patterns of temporal association among behaviours. In response to juvenile mantids, the transitions diagram is less dispersed than that in the presence of jumping spiders (Figure 4), suggesting that the degree of association between behaviours in the presence of mantids is more extreme. While most behaviours (abdominal lift, fly, groom, jump, run, stop, and turn) tend to transition to walking, we also see stronger associations between other pairs of behaviours. For example: after performing the retreat behaviour, fruit flies often either abdominal lift or turn, and flight is often followed by stopping. These observations are supported by the findings that in the absence of mantids, 12 transitions showed significant deviations from expectations (Supplement b, Table S4). In comparison, in the presence of mantids 23 transitions showed a significant deviation from expected values (Supplement b, Table S4). Interestingly, as with the spiders the log-linear model supports the non-independence of behavioural states (resid df=71, deviance=1054, $p < 0.001$), but not for the additional influence of predator state on this non-independence (resid df = 71, deviance = 72, $p=0.4$).

DISCUSSION

Prey organisms can alter their behaviour to reduce the likelihood of detection, capture or encounter with a predator [5]. For example, when predators are present, ground squirrels dedicate more time to vigilance behaviours (like scanning for a predator, see [51]) and some aquatic insects spend more time in refuges [52]. These changes in 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.

We also identified a number of (to our knowledge) undescribed behaviours of *D. melanogaster,* potentially relating to its interactions with predators. The behaviour we called "stopping" (Table 1) was observed numerous times after a direct (but failed) attack by a spider (Supplement 3 video 1). While *D. melanogaster* will spend time without any ambulatory activity (walking, running), they are almost always observed to be active (generally grooming) during these periods. However, when fruit flies performed the stopping behaviour, there was a complete lack of movement on the part of the fly, even when video was viewed at a few frames/second. When a fruit fly was "stopped", the spider had to search for the fly, irrespective of the physical proximity between the spider and the fly. In salticids, while the principal eyes have high spatial acuity [34], secondary eyes are primarily used to detect moving objects [56]. Because salticids are unable to accommodate by changing the shape of their lens, they need to extensively sample their visual field to see details in object shape and form [34,55,58]. Scanning for prey by such sampling is likely a slow process unless guided by the motion sensing peripheral eyes, giving motionless prey the advantage of staying hidden (at least for a few seconds) while in plain sight of their salticid predator. Thus, *D. melanogaster* may be using the "stopping" behaviour as a potential mechanism to reduce the likelihood of detection by the spider.

Additionally, in the presence of both predators, *D. melanogaster* substantially increase the frequency at which it performed abdominal lifts. To our knowledge, abdominal lifting has not been described in *D. melanogaster* literature before and may be relevant in an anti-predatory context. While studying courtship behaviours in female *D. 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

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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

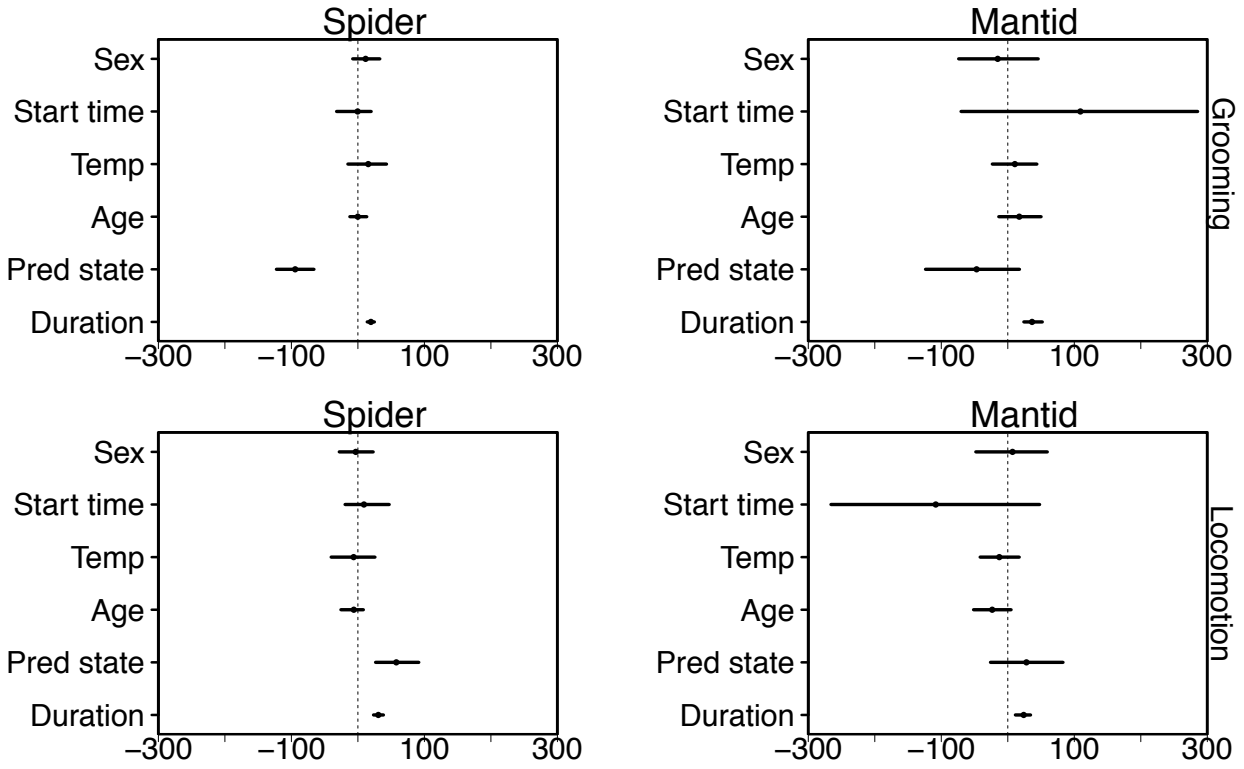

Figure S1

Addition of jumping spiders cause fruit flies to walk more and groom less, whereas the presence of mantids show 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 and locomotion) as a function of predator state (present vs absent of spiders, left panels and mantids, right panels), total duration of the assay, 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.

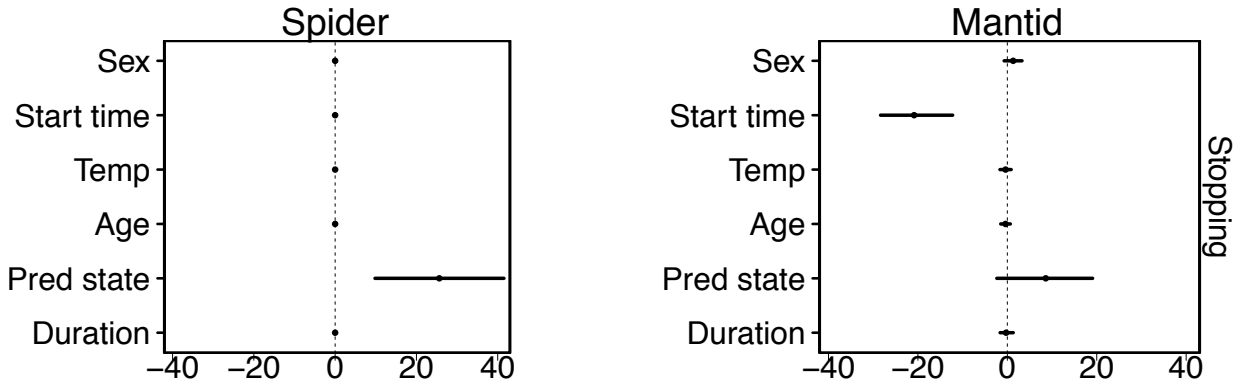

Figure S2

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), total duration of the assay, 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. 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".

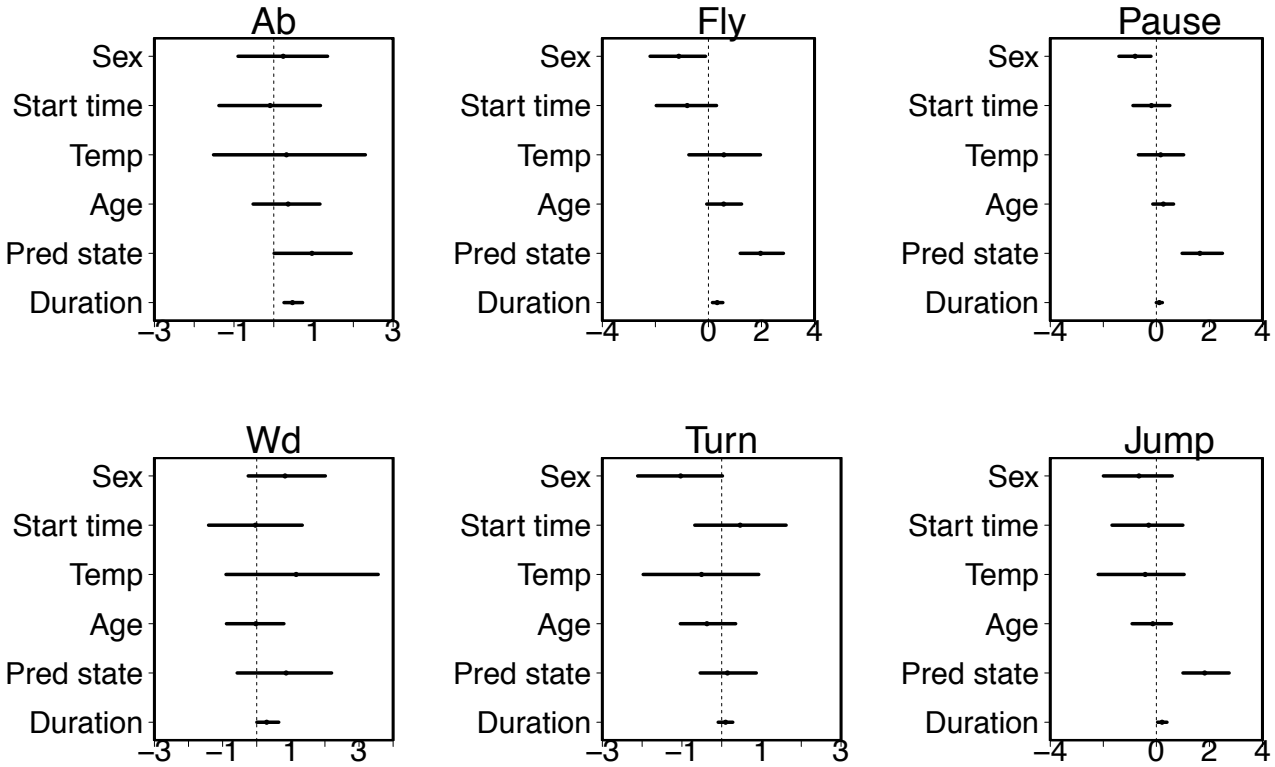

Figure S3

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 using the package MCMCglmm to visualize duration of each individual behavioural event (ab, fly, pause, wd, turn and jump) as a function of predator state (present vs absent of a spider), total duration of the assay, 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.

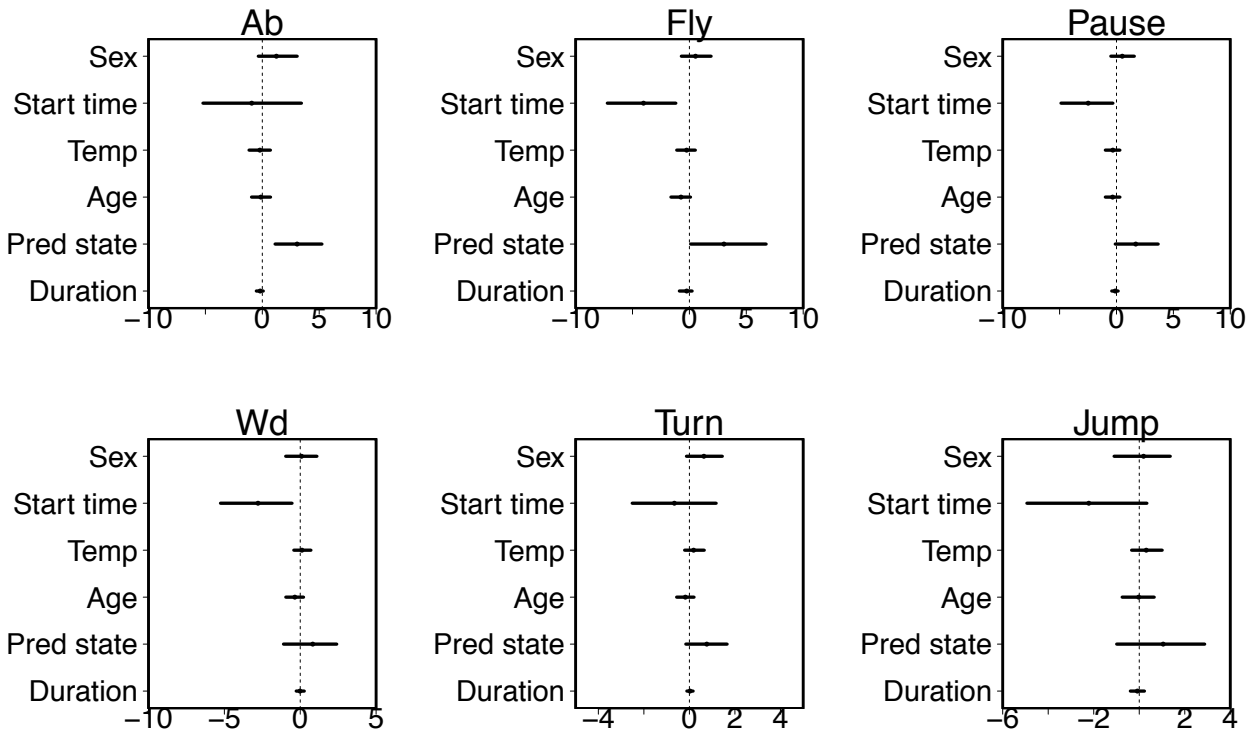

Figure S4

Fruit flies performed abdominal lifts are a higher frequency in the presence of a juvenile mantid. Here we show coefficient plots made from the output of mixed effects models using the package MCMCglmm to visualize duration of each individual behavioural event (ab, fly, pause, wd, turn and jump) as a function of predator state (present vs absent of a mantid), total duration of the assay, 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.

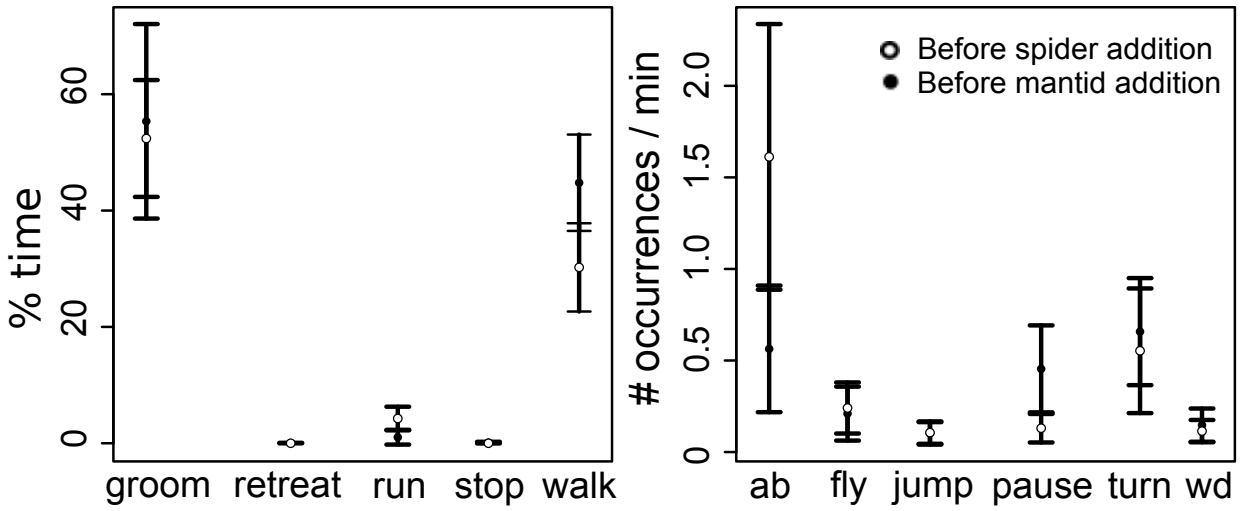

Figure S5

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.

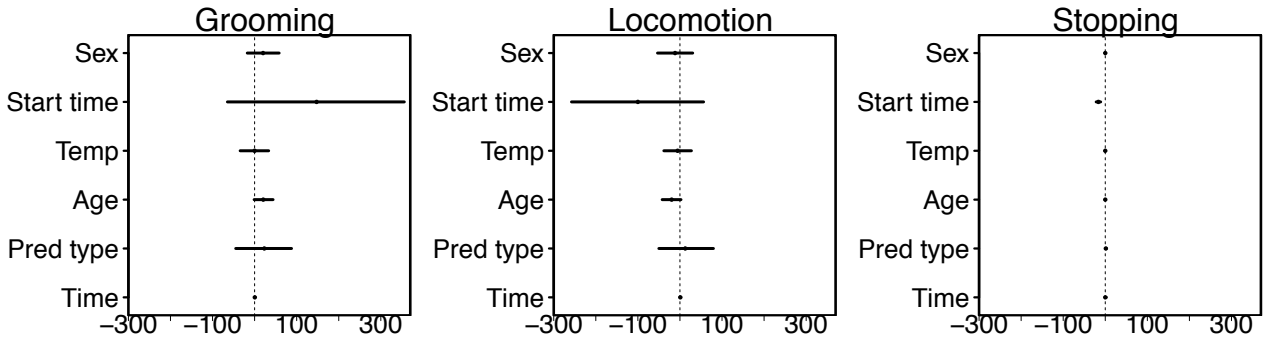

Figure S6

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

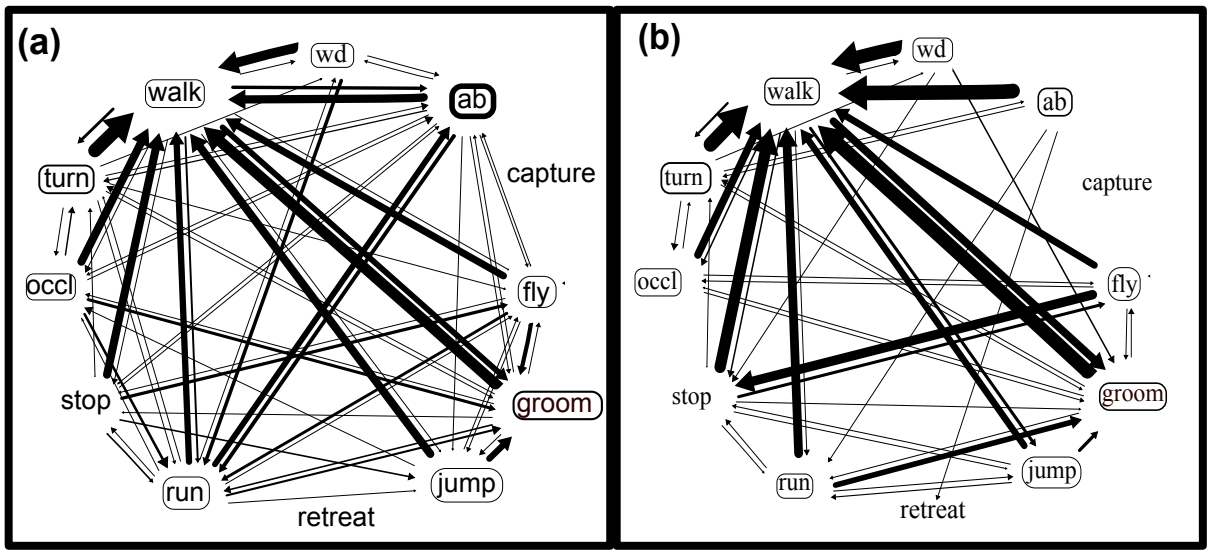

Figure S7

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