Infection avoidance behaviour in adult fruit flies is sex-specific and depends on prior exposure to a viral pathogen

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Abstract

Infection avoidance behaviours are the first line of defence against pathogenic encounters. Behavioural plasticity in response to internal or external cues can therefore generate heterogeneity in infection. We tested whether *Drosophila melanogaster* exhibits infection avoidance behaviour during foraging, and whether this behaviour is modified by prior exposure to Drosophila C Virus (DCV) and by the risk of DCV encounter. We examined two measures of infection avoidance: (1) the motivation to feed in the presence of an infection risk and (2) the preference to feed on a clean food source over a potentially infectious source. We found no clear evidence for preference of clean food sources over potentially infectious ones. However, infection avoidance was present in female fruit, which were less motivated to feed when presented with a risk of encountering DCV, but this was only the case if they had been previously exposed to this viral pathogen. We discuss the relevance of plasticity in avoidance behaviours during ecologically relevant scenarios such as foraging for host fitness and pathogen spread.

Key-words: Infection, avoidance behaviour, Drosophila, DCV, foraging

Background

Hosts vary considerably in their ability to acquire and transmit infection (Barron et al., 2015; Fellous et al., 2012; Paull et al., 2011; Susi et al., 2015; Vale and Little, 2009). Given the ubiquitous presence of pathogens and parasites in natural environments, mounting a timely and efficient immune response to all possible pathogenic challenges would be physiologically costly and ultimately ineffective. Individuals capable of reducing the probability of contacting parasites, infected conspecifics or infectious environments can therefore not only prevent the deleterious effects of infection, but also circumvent the undesirable energetic costs of immune responses, including immunopathology (Barron et al., 2015; Curtis, 2014). Avoiding infection is therefore the first line of non-immunological defence against infection(Parker et al., 2011), and it is known to occur across a broad range of host taxa, including humans (Curtis, 2014; Moore, 2013).

Like most traits, infection avoidance behaviours are likely to vary according to the context of infection, and pathogens are major drivers this context (Barron et al., 2015; Curtis, 2014; Lazzaro and Little, 2009; Moore, 2013; Vale et al., 2008; Wolinska and King, 2009). Pathogens may alter host responses in two ways. First, by altering the immuno-physiology of the host during infection, pathogens can alter host behaviour (Adamo, 2006; Adelman and Martin, 2009). Second, pathogens also modify the host external environment by increasing the likelihood of exposure to novel infections, and these external cues of infection risk are also known to influence host behavioural responses (Barron et al., 2015; Curtis, 2014). Understanding variation in infection avoidance behaviours therefore provides an important functional link between the neurological, behavioural and immunological processes that together govern the spread of disease (Adamo, 2006).

Insects are ideal systems to investigate the interplay between infection and behaviour (Adamo, 2006; Parker et al., 2010). The fruit fly *Drosophila* is especially amenable to these studies, as it is one of the best model systems for host-pathogen interactions (Neyen et al., 2014) and behavioural ecology and genetics (Dubnau, 2014; Sokolowski, 2001). One of the best-studied pathogenic interactions in *Drosophila* is the host response to systemic and

enteric infection with Drosophila C Virus (DCV) (Dostert et al., 2005; Ferreira et al., 2014). DCV is a horizontally transmitted +ssRNA virus that naturally infects the fly gut (Huszar and Imler, 2008), causing intestinal obstruction, severe metabolic dysfunction and eventually death (Chtarbanova et al., 2014). As a consequence of its pathology, female flies infected with DCV are also known to exhibit behavioural modifications, such as reduced locomotion and increased sleep (Vale and Jardine, 2015). The Drosophila-DCV interaction therefore offers a powerful system to investigate the ecological consequences that may arise from the physiological and behavioural effects of enteric viral infections.

In the present study we used a combination of controlled experimental infections and foraging choice assays, to test whether adult *D. melanogaster* are able to avoid potentially infectious environments when foraging for food, and if avoidance behaviour is modified in response to virus exposure history and to different risks of acquiring DCV infection. We find evidence for avoidance behaviours in the form of reduced motivation to feed according to the risk of infection. However, these effects were only present in female flies, indicating potentially important sexual dimorphism in infection avoidance, and were only present when females were previously exposed to DCV.

Methods

Fly and virus stocks

All flies used were from a long-term laboratory stock of Wolbachia-free *Drosophila melanogaster* Oregon R line, maintained on Lewis medium in standard conditions: 25°C, with a 16:8h light:dark cycle. Fly stocks were routinely kept on a 14-day cycle with non-overlapping generations under low larval densities. The DCV culture used in this experiment was grown in Schneider Drosophila Line 2 (DL2) as described in (Vale and Jardine, 2015). Ten-fold serial dilutions of this culture (diluted in Ringers buffer solution) were aliquoted and frozen at -80ºC for long-term storage before use.

Prior virus exposure

102 Flies used in the foraging choice assays were obtained by preparing 10
103 vials of Lewis medium and yeast containing ten mated females. Flies were
104 allowed to lay eggs for 48 hours resulting in age-matched progeny reared in
105 similar larval densities. Two to three days after eclosion, these progeny were
106 exposed to DCV via the oral route of infection (Vale and Jardine, 2015), in
107 order to test the effect of previous exposure to virus on avoidance behaviour
108 during foraging. Briefly, single-sex groups of 20 flies were placed in vials
109 containing agar previously sprayed with DCV ("exposed" to 50 µl of 10^8 viral
110 copies/ml) or the equivalent volume of Ringers buffer solution as a control
111 ("not exposed"). This procedure produced 10 replicate vials of either healthy
112 or virus-exposed male or female flies. DCV exposure using this protocol
113 typically results in ~20% mortality (Fig. S1 and (Vale and Jardine, 2015)).
114
115 *Foraging choice assays*
116 Following 5 days of virus exposure, we set up independent foraging
117 choice assays in cages - cylindrical transparent plastic containers (12 cm in
118 diameter) containing two equally spaced plastic vials of standard Lewis fly
119 medium and yeast. For each combination of "exposed" and "not exposed" male
120 or female flies, we set up two sets of cages to simulate different risks of
121 infection: a "no risk" environment, with two clean vials (sprayed with sterile
122 Ringers solution), and a "high-risk" environment where one of the vials was
123 sprayed with DCV, as described above. Six replicate 20-fly groups were
124 allocated to the "high-risk" chambers and four replicates to the "no risk"
125 chambers, resulting in a total of 40 independent foraging choice cages. Flies
126 were added to the chamber from a neutrally placed hole in the lid, and the
127 number of flies that settled on each vial was recorded every 30 minutes for six
128 hours. Care was taken to randomise the position of the cages so that the
129 orientation of the light did not influence the choice of the flies in any
130 systematic way.
131
132 *Statistical Analysis*
133 To measure infection avoidance, we took two approaches. First, we
134 hypothesised that the motivation to feed would be lower in environments
135 where the risk of infection is higher (Curtis, 2014). We therefore compared
136 the motivation to feed between the "no risk" and "high-risk" cages, measured

as the proportion of flies inside a cage that chose to feed on any of the provided food sources. We also asked whether flies that chose to feed showed any evidence of avoiding potentially infectious food sources. For this analysis we focussed on the proportion of flies choosing the clean food source over the infectious food source in the "high risk" cages. In both analyses of 'motivation to feed' and 'infection avoidance', data on the proportion of flies choosing each food source within each replicate cage were analysed with a generalised linear model assuming binomial error and logit link function, and included fly 'sex', 'previous exposure' and 'infection risk' as fixed effects. 'Replicate cage' was included as a random effect, nested within treatments. We also analysed the average motivation to feed and infection avoidance across all time points, in a model including "time" as a random effect. Treatment specific contrasts were used to test the significance of pairwise comparisons. Analyses were carried out using JMP 12 (JMP).

Results

Once inside the cages, only a fraction of flies chose either of the food sources provided, and this motivation to feed increased over time for flies in all treatment groups (χ^2_1= 11.00, p=0.001; Fig. S2). The rate at which motivation increased differed between sexes ('Time × Sex' interaction, χ^2_1= 12.47, p=0.0004), and on average female flies showed greater motivation to feed than males (χ^2_1= 5.01, p=0.025), with 67% of female and 36% of male flies making a choice to feed on any of the provided substrates during the 6 hours of observation (Fig. 1).

Across the entire six-hour observation period, the motivation to feed differed between sexes, and depended both on their previous exposure and on their current risk of infection ('Sex' × 'risk of infection' × 'Previous exposure' interaction, χ^2_1= 21.82, p<0.0001). The proportion of males choosing any food substrate did not vary with previous exposure to DCV in either high-risk (χ^2_1= 2.21, p=0.137) or no-risk environments (χ^2_1= 0.09, p=0.764; Fig. 1).

In female flies however, previous exposure and current infection risk affected the motivation to feed on the provided food sources. When there was no risk of infection (Fig. 1, light grey bars) the motivation to feed was greater

172 in females that were previously exposed to DCV than in otherwise healthy,
173 non-exposed females (χ^2_1= 104.11, p<0.001). Among females that were
174 previously exposed to infection, we found that the presence of a risk of
175 acquiring infection resulted in lower foraging effort - with just over 50% of
176 flies making the choice to feed - compared to females in cages where there
177 was no risk of acquiring infection, where over 80% of flies made the choice to
178 feed (Fig. 1; χ^2_1= 168.48, p<0.001).
179
180 Once flies had made the choice to feed on one of the provided food
181 sources, the choice between a clean and a potentially infectious food source
182 was not affected by previous exposure to DCV ('previous exposure', χ^2_1=
183 0.513, p=0.47) in either male or females ('sex', χ^2_1= 0.595, p=0.44; Fig. 2).
184
185 **Discussion**
186 The ability to detect and discriminate between clean and potentially
187 infectious environments is vital to avoid the adverse consequences of
188 infection. In this study we tested if infection avoidance behaviour in
189 *Drosophila melanogaster* is modified by its previous exposure to a viral
190 pathogen and by the risk of infection with that same pathogen when
191 encountered during foraging.
192
193 The higher motivation to feed of some female flies when the risk of infection
194 was absent (Fig. 1) suggests flies were able to identify external cues of
195 infection risk. Identifying infection cues is a general prerequisite of avoidance
196 behaviours and occurs across a wide range of different taxa. For example,
197 lobsters are known to detect and avoid virus-infected conspecifics (Behringer
198 et al., 2006); fruit flies and nematodes are capable of avoiding pathogenic
199 bacteria (Babin et al., 2014; Meisel and Kim, 2014); gypsy moth larvae are able
200 to detect and avoid virus-contaminated foliage (Parker et al., 2010); sheep
201 have been found to prefer to graze in parasite-poor patches (Hutchings et al.,
202 2007); and it is has been argued that the disgust response in humans has
203 evolved because it decreases contact with potential infection (Curtis et al.,
204 2011). It is unclear how flies are able to detect food sources contaminated
205 with a viral pathogen. In *Drosophila* and *C. elegans* avoidance of pathogenic
206 bacteria is enabled by evolutionary conserved olfactory and chemosensory

207 pathways (Babin et al., 2014; Meisel and Kim, 2014), while avoidance of
208 parasitic wasps appears to be mainly enabled by the visual sensory system
209 (Kacsoh et al., 2013). While avoiding virus infected conspecifics is probably
210 driven by visual cues of infection (Behringer et al., 2006), it remains unclear
211 how virus-contaminated environments may be detected by *Drosophila*.
212
213 Infection avoidance, measured as a reduced motivation to feed, was
214 clearest when flies had been previously exposed to infection (Fig. 1). In
215 addition to responding to external cues of infection, internal physiological
216 cues therefore also modify avoidance behaviour. Behavioural modifications
217 due to infection are widely reported among animals (Barber and Dingemanse,
218 2010; Moore, 2013), and can be classified into (i) parasitic manipulation that
219 enhances parasite transmission (Moore, 2013) (ii) sickness behaviours that
220 benefit the host by conserving energetic resources during infection (Adelman
221 and Martin, 2009), or (iii) side-effects of pathogenicity that do not benefit the
222 host or the parasite (Barber and Dingemanse, 2010). Female flies infected
223 with DCV are known to experience increased lethargy and sleep (Vale and
224 Jardine, 2015), so these effects could also explain the reduced feeding activity
225 we detected in female flies that had been previously exposed to DCV. Another
226 potential explanation for reduced motivation to feed in previously exposed
227 flies is infection-induced anorexia (Ayres and Schneider, 2009), a commonly
228 described sickness behaviour (Adelman and Martin, 2009). However, it is
229 unlikely that a lower motivation feed is simply a symptom of a "sick" fly,
230 because it varied according risk of infection, and even reached 80% in
231 exposed flies when foraging in a 'no risk' environment (Fig. 1). This suggests
232 that flies are actively avoiding contact with the potentially contagious food
233 source by lowering their foraging effort.
234
235 The fact that only female flies demonstrated avoidance is an indication
236 that any potentially adaptive effects of avoiding infection may be related to
237 oviposition, which coincides with feeding. For flies previously exposed to DCV,
238 avoiding infection would not confer substantial direct benefits given the
239 physiological and behavioural costs of this infection (Arnold et al., 2013;
240 Chtarbanova et al., 2014; Vale and Jardine, 2015), but would however reduce
241 the exposure of future offspring to infection. While flies previously exposed to

242 DCV do not appear to immune primed following an initial viral exposure
243 (Longdon et al., 2013), our results point to a sort of behavioural priming,
244 where females previously exposed to infection avoid foraging in potentially
245 infectious environments.
246
247 **Conclusions**
248 Using a combination of experimental infections and behavioural
249 assays, we find evidence for avoidance behaviours in *Drosophila* in the form of
250 reduced motivation to feed, which was most pronounced when flies were
251 faced with an increased risk of encountering an infectious food source.
252 However, these effects were only present in female flies, indicating potentially
253 important sexual dimorphism in infection avoidance, and were only present
254 when females were previously exposed to DCV. Understanding how avoidance
255 behaviours may vary is therefore important for our understanding of how
256 disease will spread in natural populations (Barron et al., 2015), and more
257 broadly how pathogens might evolve in response to variation in host
258 responses to infection (Boots and Bowers, 1999; McLeod and Day, 2015).
259
260 **Competing interests**
261 The authors declare that they have no competing interests.
262
263 **Author contributions**
264 PFV conceived the study. PFV and MDJ designed the experiment. MDJ carried
265 out the experimental work. PFV analysed the data, wrote the manuscript and
266 provided all research consumables.
267
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276 **Figure legends**

277

278 **Fig. 1.** The motivation to feed, measured as the proportion of flies in the cage
279 that fed on any of the provided food sources. Single sex-groups of flies that
280 had either been previously exposed to DCV or to a sterile inoculum were
281 tested in a no-risk environment (choice between two clean vials; light grey) or
282 a high-risk environment (choice between a clean vial and a DCV-contaminated
283 vial; dark grey). Data are means ± SE.

284

285 **Fig. 2.** Infection avoidance, measured as the proportion of flies in the cage that
286 preferred to settle on the clean food source relative to the DCV-contaminated
287 food source. Single sex-groups of flies that had either been previously exposed
288 to DCV or to a sterile inoculum were tested in a high-risk environment (choice
289 between a clean vial and a DCV-contaminated vial). Data are means ± SE.

290

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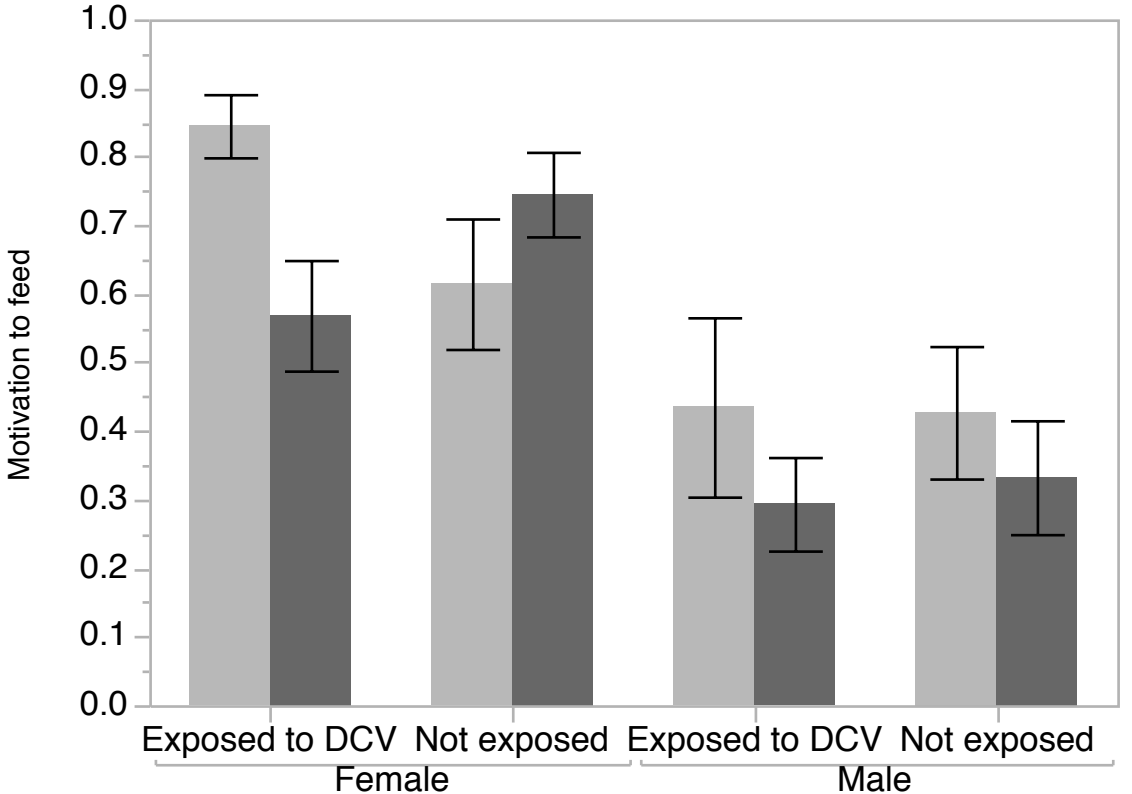
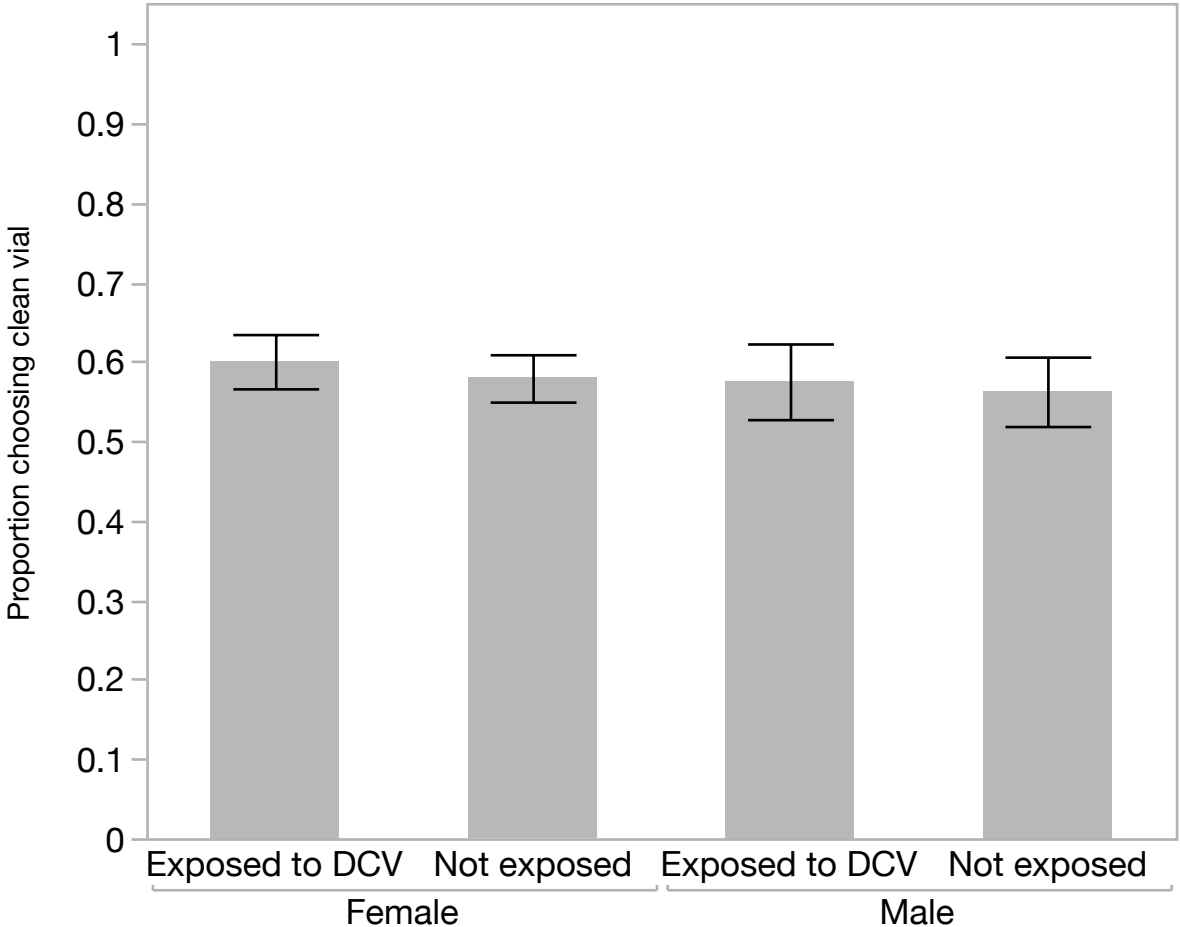
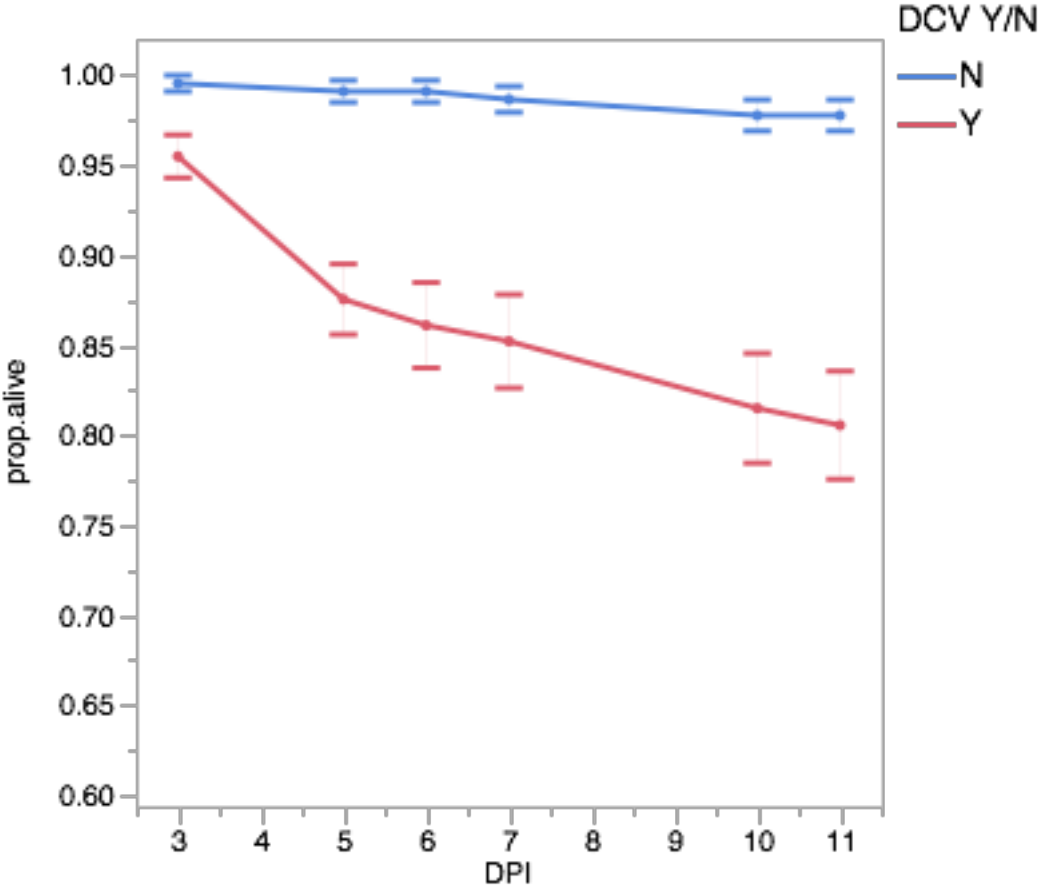
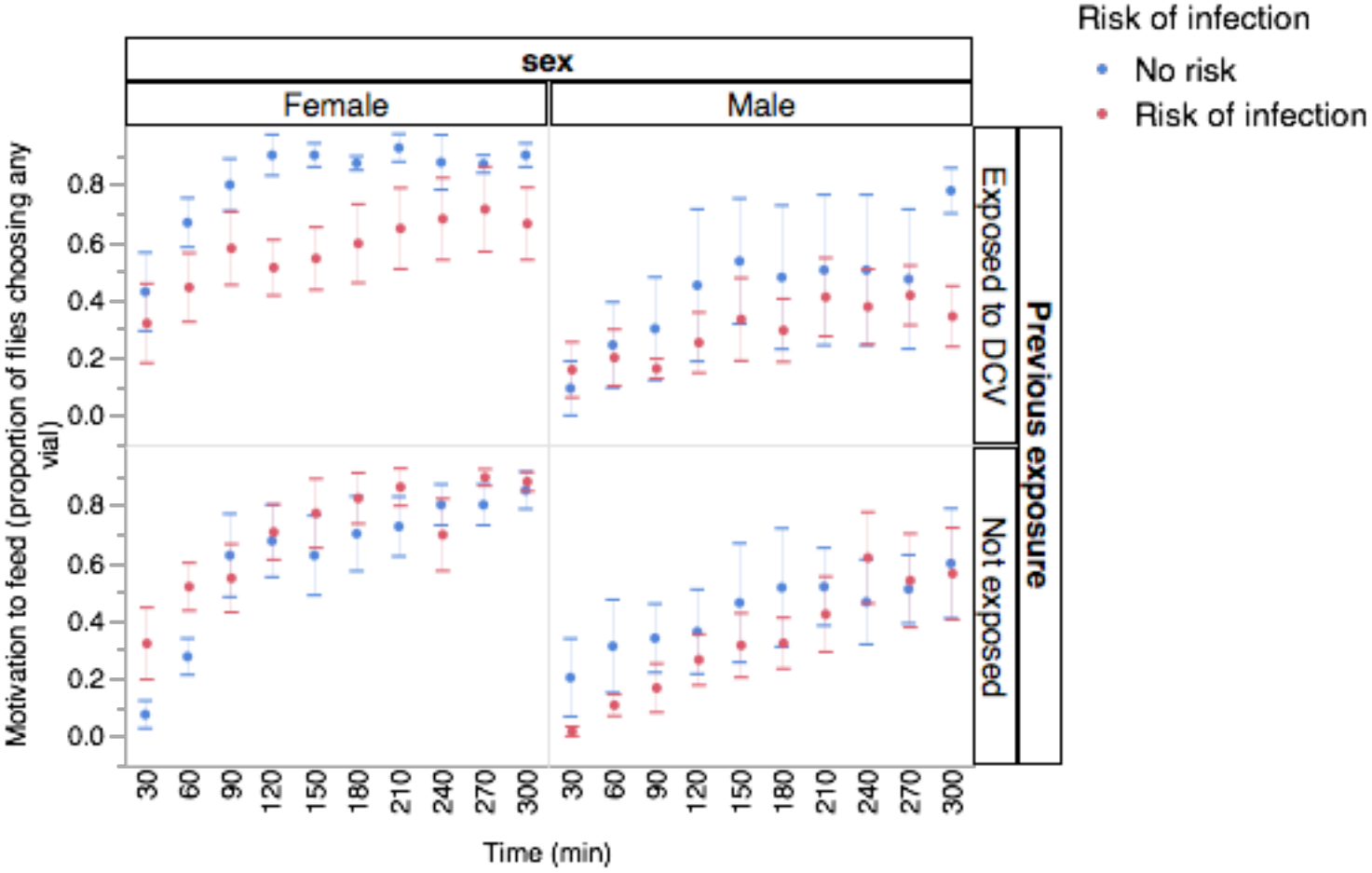
399


Motivation to feed
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Exposed to DCV
Not exposed
Exposed to DCV
Not exposed
Female
Male


Proportion choosing clean vial
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Exposed to DCV
Not exposed
Exposed to DCV
Not exposed
Female
Male


DCV Y/N
N
Y
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
prop.alive
3
4
5
6
7
8
9
10
11
DPI


Risk of infection
No risk
Risk of infection
sex
Female
Male
Previous exposure
Exposed to DCV
Not exposed
Motivation to feed (proportion of flies choosing any vial)
0.0
0.2
0.4
0.6
0.8
30
60
90
120
150
180
210
240
270
300
Time (min)