1 **Title: Desiccation stress acts as cause as well as cost of dispersal in *Drosophila***
2 ***melanogaster***

3 **Authors:** Abhishek Mishra[1], Sudipta Tung[2], V.R. Shree Sruti[3], P.M. Shreenidhi[4], and Sutirth
4 Dey*

5 **Affiliation:**

6 Population Biology Laboratory, Biology Division, Indian Institute of Science Education and
7 Research-Pune, Dr. Homi Bhabha Road, Pune, Maharashtra, India, 411 008
8
9 1 Present Address: Department of Microbiology, The Ohio State University, Columbus, OH,
10 USA, 43210
11
12 2 Present Address: Department of Biology, Ashoka University, Sonipat, Haryana, India,
13 131029

14 3 Present Address: School of Education, Azim Premji University, Bengaluru, Karnataka,
15 India, 562125

16 4 Present Address: Department of Biology, Washington University, St. Louis, MO, USA,
17 63130

18

19 ***Corresponding author:**

20 Sutirth Dey
21 Professor, Biology Division
22 Indian Institute of Science Education and Research, Pune
23 Dr. Homi Bhabha Road, Pune, Maharashtra, India 411 008
24 Tel: +91-20-25908054

25 Email: s.dey@iiserpune.ac.in

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30 **Data Accessibility Statement:** Data will be deposited in Dryad if accepted.

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Abstract

1. Environmental stress is one of the important causes of biological dispersal. At the same time, the process of dispersal itself can incur and/or increase susceptibility to stress for the dispersing individuals. Therefore, in principle, stress can serve as both a cause and a cost of dispersal.

2. Desiccation stress is an environmentally relevant stress faced by many organisms, known to shape their population dynamics and distribution. However, the potentially contrasting roles of desiccation stress as a cause and a cost of dispersal have not been investigated. Furthermore, while desiccation stress often affects organisms in a sex-biased manner, it is not known whether the desiccation-dispersal relationship varies between males and females.

3. We studied the role of desiccation stress as a cause and cost of dispersal in a series of experiments using *D. melanogaster* adults in two-patch dispersal setups. We were interested in knowing whether (a) dispersers are the individuals that are more susceptible to desiccation stress, (b) dispersers pay a cost in terms of reduced resistance to desiccation stress, (c) dispersal evolution alters the desiccation cost of dispersal, and (d) females pay a reproductive cost of dispersal. For this, we modulated the degree of desiccation stress faced by the flies as well as the provision of rest following a dispersal event.

4. Our data showed that desiccation stress served as a significant cause of dispersal in both sexes. Further investigation revealed an increase in both male and female dispersal propensity with increasing desiccation duration. Next, we found a male-biased cost of dispersal in terms of reduced desiccation resistance. This trend was preserved in dispersal-selected and non-selected controls as well, where the desiccation cost of dispersal in females was very low compared to the males. Finally, we found that the females instead paid a significant reproductive cost of dispersal.

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56   5. Our results highlight the complex relationship between desiccation stress and dispersal,

57   whereby desiccation resistance can show both a positive and a negative association with

58   dispersal. Furthermore, the sex differences observed in these trait associations may translate

59   into differences in movement patterns, thereby giving rise to sex-biased dispersal.

60

61   **Keywords:** Dispersal propensity, desiccation resistance, life history, dispersal syndrome,

62   fecundity, movement ecology, dispersal evolution, spatial selection

63

1. Introduction

Biological dispersal is often driven by numerous biotic and abiotic causes that promote movement across space (Matthysen 2012). However, the very process of movement can be costly to the dispersing organisms in several ways (Bonte *et al.* 2012). Investigating the causes and costs of dispersal can therefore help understand the constraints faced by individual organisms (Ronce & Clobert 2012), as well as their potential effects on the population- and community-level consequences of dispersal (Bowler & Benton 2005).

Since dispersal is a key life-history trait in individuals (Bonte & Dahirel 2017), one possible way to decipher its causes and costs is by studying its associations with other life-history and behavioural traits. Collectively known as a 'dispersal syndrome' (Ronce & Clobert 2012), these dispersal-trait associations have been documented in several taxa (Stevens *et al.* 2014; Legrand *et al.* 2016; Comte & Olden 2018; Tung *et al.* 2018a). While these trait correlations can help us understand the underlying physiological mechanisms and constraints of dispersal, they are often contingent on the study environment and population history. This is because trait associations change rapidly and significantly if the environment changes, or if the population undergoes evolutionary changes (Chippindale, Ngo & Rose 2003; Jessup & Bohannan 2008; Mishra *et al.* 2018a). Moreover, dispersal may be modulated by many causes at once (Matthysen 2012; Legrand *et al.* 2015), and incur several simultaneous costs to the individuals (Roff 1977; Gros, Hovestadt & Poethke 2008; Bonte *et al.* 2012). Taken together, this makes a thorough investigation of dispersal-trait associations difficult under natural conditions. Therefore, one possibility is to study populations with a known history under a simplified environment to understand how a particular trait association (and hence, the dispersal syndrome) is shaped.

87   Desiccation stress is one of the factors that can greatly influence dispersal. Not only is it one

88   of the most commonly faced environmental stress for numerous taxa (Black & Pritchard

89   2002; Holmstrup, Hedlund & Boriss 2002; Kranner *et al.* 2008; Holzinger & Karsten 2013),

90   it is also one of the first signs of an unfavourable environment, as the stress due to lack of

91   water sometimes precedes lack of other resources such as food (Karan & Parkash 1998;

92   Hoffmann & Harshman 1999). Understandably, desiccation not only affects the physiology

93   of individual organisms (e.g. Gibbs, Chippindale & Rose 1997; Folk & Bradley 2004;

94   Bazinet *et al.* 2010), but is also an important determinant of species distributions (e.g.

95   Kellermann *et al.* 2009; Rajpurohit, Nedved & Gibbs 2013). Furthermore, organisms'

96   responses to desiccation stress are particularly important in the context of climate change and

97   its biological implications (Hoffmann *et al.* 2003; Tuba, Slack & Stark 2011; Van

98   Heerwaarden & Sgrò 2014). Given that dispersal often serves as the first line of defence

99   against unfavourable environments for many taxa (Gerber & Kokko 2018; Riotte-Lambert &

100 Matthiopoulos 2020), it is crucial to investigate the relationship between biological dispersal

101 and desiccation stress.

102 Desiccation stress can potentially act as both a cause and a cost of dispersal. A high

103 desiccation stress may drive individuals away from an area, while at the same time, the very

104 process of movement can incur desiccation stress to the dispersers. Since males and females

105 in sexually dimorphic species often differ in the amount of body resources and their

106 partitioning along the survival-reproduction axis (Rantala & Roff 2007; Wilkin & Sheldon

107 2009; Maklakov & Lummaa 2013), differences in their desiccation profiles are commonplace

108 (Jill & Daniel 2003; Matzkin, Watts & Markow 2007; Lyons *et al.* 2014). Similarly, many

109 species exhibit 'sex-biased dispersal', a possible reflection of asymmetric cost-benefit

110 outcomes of dispersal between the sexes (Trochet *et al.* 2016; Li & Kokko 2019). While the

111 relationship among environmental stress, dispersal and sex have been recently discussed

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112   (Gerber & Kokko 2018), sex differences in the dispersal-desiccation relationship have

113   typically not been studied. This is not surprising given that, investigations into sex

114   differences in dispersal syndromes are relatively rare in the dispersal literature (but see

115   Legrand *et al.* 2016; Mishra *et al.* 2018a). The presence of pervasive sex differences in the

116   life-history and behaviour literature leads us to anticipate some sex differences in the

117   relationship between dispersal and desiccation stress as well. Especially in terms of dispersal

118   costs, it would be interesting to see how the desiccation stress incurred during movement

119   compares with other dispersal-related fitness costs such as female fecundity (Roff &

120   Fairbairn 2007; Guerra 2011).

121   Here, we investigate the relationship between desiccation stress and dispersal, as well as the

122   associated sex differences, using populations of the common fruit fly (*Drosophila*

123   *melanogaster*) under controlled environmental conditions. Interestingly, both a positive and a

124   negative association of desiccation stress with dispersal has already been reported in *D.*

125   *melanogaster* (Mishra *et al.* 2018a), thus making it a suitable system to delineate how the

126   desiccation-dispersal relationship is shaped. Specifically, we asked the following questions:

127   (1) Does desiccation stress act as a cause of dispersal in males and females? (2) Is desiccation

128   stress a cost of dispersal in males and females? (3) Does dispersal evolution alter the

129   desiccation cost of dispersal in either sex, and (4) Do females experience a fecundity cost of

130   dispersal? Our results showed that desiccation stress acts as a significant cause for dispersal

131   for both sexes. However, desiccation stress emerged as a cost of dispersal largely in the

132   males, and was not altered by dispersal evolution. Finally, while the females paid a negligible

133   desiccation cost of dispersal, they experienced a significant cost of dispersal in terms of their

134   fecundity. We discuss these results in the context of *Drosophila* physiology, along with their

135   implications for dispersal patterns.

136

137   **2. Methods**

138   2.1 Fly populations

139   We used large, outbred laboratory populations (breeding size ~2400 individuals) of *D.*

140   *melanogaster* for all the experiments in this study. The ancestry of these populations can be

141   traced back to the IV lines, which were wild-caught in South Amherst, MA, USA (Ives

142   1970). The single-generation experiments in this study were conducted using a baseline

143   population named DB_4 (Sah, Salve & Dey 2013; Mishra *et al.* 2020). In addition, we used

144   four dispersal-selected populations (namely, VB_{1-4}) and their corresponding controls, the non-

145   selected populations (VBC_{1-4}), for one experiment. Due to the ongoing selection for higher

146   dispersal every generation, the VB populations have evolved a higher dispersal propensity

147   and ability (Tung *et al.* 2018b), as well as lower desiccation resistance (Mishra *et al.* 2018a),

148   compared with the VBC populations. All the populations were maintained in discrete-

149   generation cycles under uniform environmental conditions of 25 °C temperature and 24-h

150   light.

151

152   2.2 Dispersal setup

153   Following previous studies (Mishra *et al.* 2018a; Tung *et al.* 2018b), we used a two-patch

154   dispersal setup for observing fly dispersal. Each dispersal setup comprised a *source* container,

155   a *path* tube and a *destination* container (Fig. 1). In this setup, all the flies for a given

156   treatment/group are first introduced into the *source* container, which opens into a transparent

157   plastic tube (internal diameter ~1 cm) that serves as the *path*. The other end of the path tube

158   leads into the *destination* container, thereby allowing the dispersal of flies from the *source* to

159   the *destination* container through the *path*, for a fixed duration. Depending on the experiment,

160   the size of the *source* and *destination* containers, as well as the length of the *path* tube, can be

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161 customized. A single experiment typically involves multiple such dispersal setups,

162 maintained under uniform environmental conditions. At the end of a dispersal run, these

163 dispersal setups are dismantled, and the flies found in each part (*source/path/destination*) are

164 used as per the experimental requirements.

165

166 <u>2.3 Experiments</u>

167 We carried out a series of experiments to address various questions related to causes and

168 costs of dispersal. The protocols, type of data obtained and the statistical analyses are

169 presented separately for each experiment below.

170 *2.3.1 Experiment 1: Desiccation stress as cause vs. cost of dispersal*

171 We first examined whether desiccation stress acts as a cause and emerges as a cost of

172 dispersal in *D. melanogaster*. For this, we started with ~19,200 age-matched (12-day-old

173 from egg collection) adult flies from the DB_4 population that were reared under identical

174 conditions of *ad libitum* food and water. Cylindrical, translucent plastic containers (~1.5 L

175 volume) were used as *source* and *destination*, along with a *path* length of 6 m, to assemble

176 two-patch dispersal setups (described in section 2.2). Batches of the aforementioned DB_4

177 individuals were then introduced into eight such dispersal setups (~2400 individuals per

178 setup) and allowed to disperse for 5 h. By modulating two factors, i.e. presence of agar-based

179 food (banana-jaggery medium) in the *source* container, and the provision of rest to flies after

180 the dispersal run, we devised three scenarios (Fig. 1A, see explanation in next paragraph): (a)

181 *Cause* scenario, where we could identify whether desiccation stress was a cause of dispersal,

182 (b) *Control* scenario, where desiccation stress was expected to be neither a cause nor a cost of

183 dispersal, and (c) *Cost* scenario, where we could identify whether desiccation stress was a

184 cost of dispersal (Fig. 1A). In each of the three scenarios, the flies that completed dispersal

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185   from the *source* to the *destination* were termed as dispersers (D), whereas the flies that were

186   found inside the *source* container were termed as non-dispersers (ND). The flies found in the

187   path at the end of the dispersal run were not used in this experiment.

188   In the *Cause* scenario, there was no food or water in the *source*, making desiccation stress a

189   likely driver of dispersal away from the source. After the dispersal event, we collected the

190   ND and D flies separately and provided them a 2-day rest with *ad libitum* food and water, so

191   that the D flies could recuperate any energy costs of dispersal run. Thereafter, we assayed

192   200 ND and 200 D flies (100 males+100 females each) for their desiccation resistance

193   (Supplementary Text S1.1), to assess whether they differed in terms of their inherent

194   desiccation sensitivity (Fig. 1A: *Cause* scenario). Here the assumption is that the rest of 2

195   days is sufficient to ameliorate any negative effects on desiccation sensitivity (similar to

196   Mishra *et al.* 2018a).

197   In the *Control* scenario, we provided agar-based banana-jaggery medium in the *source*

198   container during the dispersal run, thereby removing desiccation stress as a possible driver of

199   dispersal. Similar to the *Cause* scenario, the dispersal event was followed by a 2-day rest to

200   both ND and D flies, to offset any energy costs of dispersal (Fig. 1A: *Control* scenario).

201   Subsequently, we compared the desiccation resistance of 200 ND and 200 D flies, to

202   ascertain if there were any unaccounted-for differences between them, i.e. other than those

203   detected in *Cause* and *Cost* scenarios.

204   The *Cost* scenario was complementary to the *Cause* scenario. Here, we provided banana-

205   jaggery medium in the *source* container, thereby removing desiccation stress as a cause of

206   dispersal, but did not allow any rest after dispersal. As above, we then compared the

207   desiccation resistance of 200 ND and 200 D flies, with any difference attributed to the energy

208   costs of dispersal (Fig. 1A: *Cost* scenario).

209   The statistical analyses for this experiment, as well as those described in subsequent sections,

210   were carried out in R v4.0.3 (R Core Team 2020). Here, the desiccation data from

211   Experiment 1 were analysed together in a single mixed-model GLM using the 'lmer' function

212   from the 'lme4' package v1.1-25 (Bates *et al.* 2015), with *scenario* (*Cause*/*Control*/*Cost*),

213   *dispersal* (ND/D) and *sex* (male/female) as the fixed factors. As the flies were assayed in

214   single-sex groups of 10 individuals within a vial (Supplementary Text S1.1), we included *vial*

215   *identity* (1–10) as a random factor that was nested within the *scenario × dispersal × sex*

216   interaction. Following a Type III analysis of deviance to ascertain the significance of the

217   fixed factors and their interactions in GLM via the 'Anova' function in 'car' package v3.0-10

218   (Fox & Weisberg 2019), we carried out the relevant pairwise comparisons using the 'pairs'

219   function in the 'emmeans' package v1.5.2-1 (Lenth 2020). Cohen's d was used as a measure

220   of effect size for significantly different pairs of means, with the effect interpreted as large,

221   medium, and small for $d \geq 0.8$, $0.8 > d \geq 0.5$, and $d < 0.5$, respectively (Cohen 1988).

222

223   *2.3.2 Experiment 2: Effect of desiccation duration on dispersal*

224   Here, we investigated how dispersal changes with the duration of desiccation stress. For this,

225   we segregated age-matched (12-day-old from egg collection) DB_4 flies into multiple groups

226   of 120 individuals (60 males + 60 females) that were subjected to varying durations of

227   desiccation stress (0, 1, 2, 3, 4, and 5 h) before being subjected to dispersal assay in separate

228   dispersal setups (Fig. 1B). The *source* here was a 100-mL glass flask without any food or

229   water, the *path* length was 2 m, and the destination was a 250-mL plastic bottle. The dispersal

230   assay lasted for 2 h. Following a previous protocol (Mishra *et al.* 2018b; Mishra, Chakraborty

231   & Dey 2020; Mishra *et al.* 2020), the experiment was carried out over 10 consecutive days

232   with a fresh set of age-matched flies every day. This allowed us to assay one replicate of

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233 every desiccation treatment each day, yielding 10 replicates blocked by *day*. In total, 6000

234 flies (5 desiccation treatments × 2 sexes × 10 days × 60 flies treatment^{-1} sex^{-1} day^{-1}) were

235 assayed for this experiment. From the dispersal assay, we collected data on dispersal

236 propensity (i.e. the fraction of flies that dispersed from the source: Friedenberg 2003;

237 Supplementary Text S1.2). To account for any day-to-day micro-environmental variation, we

238 used *day* as a random blocking factor in the analysis. Therefore, the dispersal propensity data

239 were analysed in a mixed model binomial GLM (with logit link function) using the 'glmer'

240 function from the 'lme4' package v1.1-25 (Bates *et al.* 2015), with *desiccation duration* (0, 1,

241 2, 3, 4, and 5 h) and *sex* (male and female) as fixed factors, and *day* (1–10) as the random

242 factor. Following analysis of deviance via 'Anova' function in 'car' package v3.0-10 (Fox &

243 Weisberg 2019), appropriate pairwise comparisons were carried out using the 'pairs' function

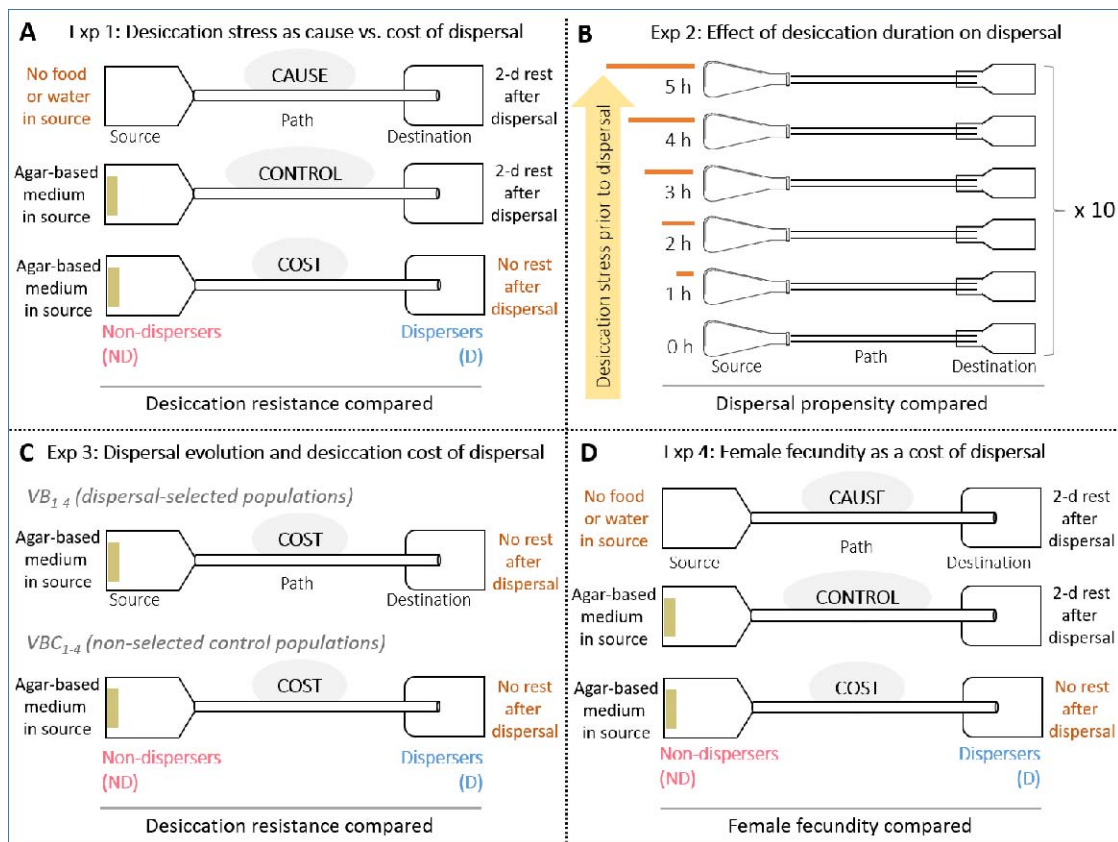244 in 'emmeans' package v1.5.2-1 (Lenth 2020).

Fig. 1: Schematics of the experimental design. (A) Experiment 1 investigated the role of desiccation

stress as a cause vs. cost of dispersal. Using a *source-path-destination* setup, age-matched flies from an outbred baseline population (DB$_4$) were segregated into non-dispersers (ND) and dispersers (D) under three scenarios: *Cause* (no food or water in *source*; rest provided after dispersal run), *Control* (agar-based banana-jaggery medium in *source*; rest provided after dispersal run), and *Cost* (agar-based banana-jaggery medium in *source*; no rest provided after dispersal run). ND and D flies within each scenario were then assayed for their desiccation resistance. **(B)** Experiment 2 further examined the role of desiccation stress as a cause of dispersal. Groups of age-matched flies from DB$_4$ population were subjected to different durations of desiccation stress (0–5 h) before being subjected to dispersal assay. **(C)** Experiment 3 investigated whether the desiccation cost of dispersal differs between populations selected for higher dispersal (VB$_{1-4}$) and their non-selected controls (VBC$_{1-4}$). Desiccation resistance of all eight population blocks was compared under the *Cost* scenario similar to Experiment 1. **(D)** Experiment 4 examined the role of female fecundity as a cause vs. cost of dispersal. Here, female ND and D flies for the three scenarios (*Cause*, *Control*, and *Cost*) were assayed for their fecundity.

245

246   *2.3.3 Experiment 3: Dispersal evolution and desiccation cost of dispersal*

247   Here, we used dispersal-selected populations (VB$_{1-4}$), which have a higher dispersal

248   propensity and travel longer distances (Tung *et al.* 2018b), as well as a lower desiccation

249   resistance, than their non-selected controls (VBC$_{1-4}$) (Mishra *et al.* 2018a). In this experiment,

250   we investigated whether the VB and VBC populations differ in their desiccation cost of

251   dispersal. This would help determine if selection for dispersal under desiccated conditions

252   has altered the magnitude of proximate cost paid by dispersers. We subjected ~2400 age-

253   matched individuals per population block (1–4) of each population type (VB/VBC) to

254   segregation into ND and D individuals under the *Cost* scenario as described in section 2.3.1

255   (Fig. 1C). Thereafter, we assayed 100 males and 100 females (in groups of 10

256   individuals/vial) from each of the eight populations (VB$_{1-4}$ and VBC$_{1-4}$) for their desiccation

257   resistance (Supplementary Text S1.1). The entire desiccation resistance data were analysed

258   using a mixed-model GLM with the 'lmer' function in 'lme4' package v1.1-25 (Bates *et al.*

259   2015), with *dispersal selection* (VB/VBC), *dispersal* (ND/D) and *sex* (male/female) as fixed

260   factors, and *population block* (1–4) and *vial identity* (1–10) as random factors. Here, *vial*

261   *identity* was nested inside the *dispersal selection × dispersal × sex × population block* term.

262   Following the GLM, we used the 'Anova' function in 'car' package v3.0-10 (Fox &

263   Weisberg 2019) for analysis of deviance, and subsequently, the 'pairs' function in 'emmeans'

264   package v1.5.2-1 (Lenth 2020) for relevant pairwise comparisons.

265

266   *2.3.4 Experiment 4: Female fecundity as cause vs. cost of dispersal*

267   This experiment aimed to examine whether females paid a dispersal cost in terms of their

268   fecundity. The female flies in this experiment were from the same ND and D groups of flies

269   that were segregated in Experiment 1, giving rise to: (a) *Cause* scenario, defined by the lack

270   of suitable oviposition site in *source* container, (b) *Control* scenario, with suitable oviposition

271   surface (i.e. banana-jaggery medium) in the *source* and provision of rest after dispersal run,

272   and (c) *Cost* scenario, where no rest is provided and flies were assayed for their fecundity

273   immediately after dispersal (Fig. 1D). We counted the female fecundity as the number of

274   eggs laid over a 12-h period, with the ND and D flies for each scenario assayed together (see

275   Supplementary Text S1.3 for details). The entire fecundity data were analysed together with a

276   quasi-Poisson GLM (with log link function) using the 'glm' function in 'stats' package v4.0.3

277   (R Core Team 2020), with *scenario* (*Cause*, *Control*, and *Cost*) and *dispersal* (ND and D) as

278   the fixed factors. As above, we used the 'Anova' function in 'car' package v3.0-10 (Fox &

279   Weisberg 2019) for analysis of deviance, and the 'pairs' function in 'emmeans' package

280   v1.5.2-1 (Lenth 2020) for relevant pairwise comparisons.

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3. Results

3.1 Desiccation stress as a cause vs. cost of dispersal

Desiccation resistance data from Experiment 1 showed a significant *scenario × dispersal × sex* interaction ($\chi^2 = 7.20$, p = 0.027). Analysis of pairwise differences for this interaction revealed a number of results (Supplementary Text S2.1). First, there was no difference in the desiccation resistance of dispersers vs. non-dispersers in the *Control* scenario ($p_{males} = 0.16$; $p_{females} = 0.34$) (Fig. 2B, 2E). This was expected, as all these flies had access to *ad libitum* food and water in the source container, as well as a 2-day rest after the dispersal event. Second, dispersers in the *Cause* scenario had a lower desiccation resistance than non-dispersers ($p_{males} = 0.006$, d = 1.63 (large); $p_{females} = 0.005$, d = 1.10 (large)) (Fig. 2A, 2D). This implies that desiccation stress likely served as a cause of dispersal in both sexes. Third, while males experienced a cost of dispersal in terms of their desiccation resistance ($p < 10^{-4}$, d = 1.21 (large)), no such cost was seen in females (p = 0.86) (Fig. 2C, 2F).
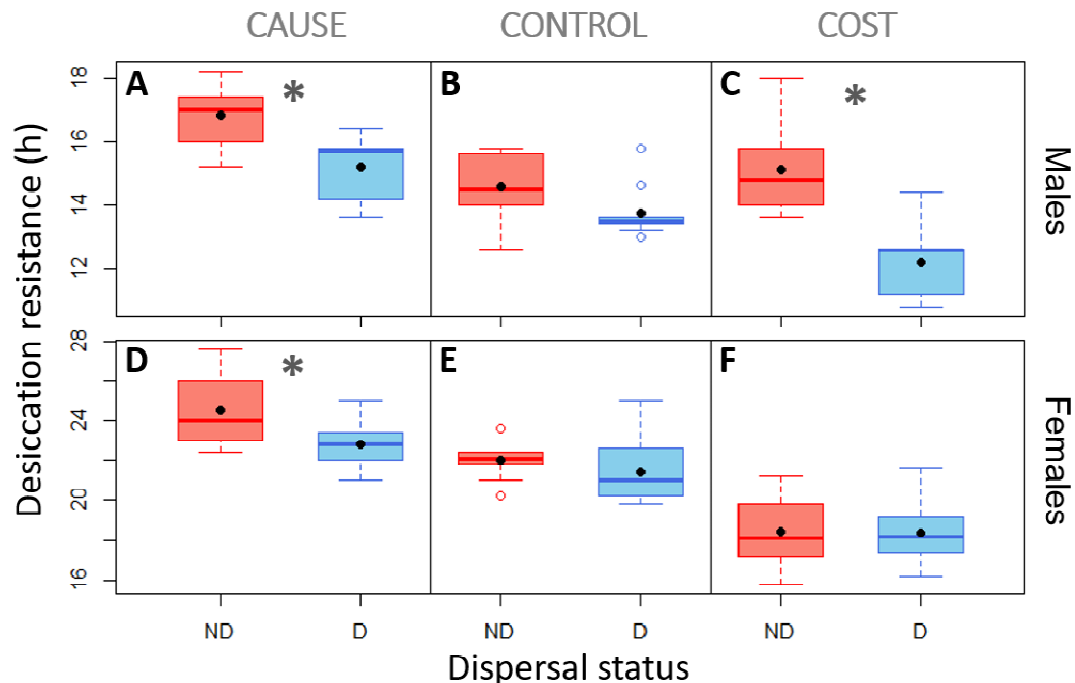

Fig. 2: Desiccation stress as cause vs. cost of dispersal (Experiment 1). Desiccation resistance for

non-disperser (ND) and disperser (D) flies from an outbred, baseline population (DB$_4$), under three scenarios: *Cause*, *Control*, and *Cost*. Data for males and females are presented in the top and bottom rows, respectively. Edges of the boxplots represent 25th and 75th percentiles of the data. The black dots represent means and the lines inside box represent medians. Asterisks (*) indicate a significant difference (p < 0.05) between ND and D flies within a given panel. Note that the scales on Y-axis differ between the males and the females. See Supplementary Text S2.1 for the exact p values.

296

297   ### 3.2 Desiccation stress as a cause of dispersal in both sexes

298   The role of desiccation stress as a cause of dispersal was further investigated in Experiment 2.

299   Analysis of data from this experiment revealed that the *desiccation duration × sex* interaction

300   was significant ($\chi^2 = 17.10$, p = 0.004), indicating an asymmetric effect of desiccation

301   duration on dispersal propensity of males and females. However, pairwise comparisons

302   revealed an increasing trend of dispersal propensity with longer durations of desiccation

303   stress in both sexes, with a somewhat greater effect observed in males (Fig. 3)

304   (Supplementary Text S2.2). Therefore, the results from both Experiments 1 and 2 suggested

305   that desiccation stress served as a cause of dispersal in both sexes, with longer durations of

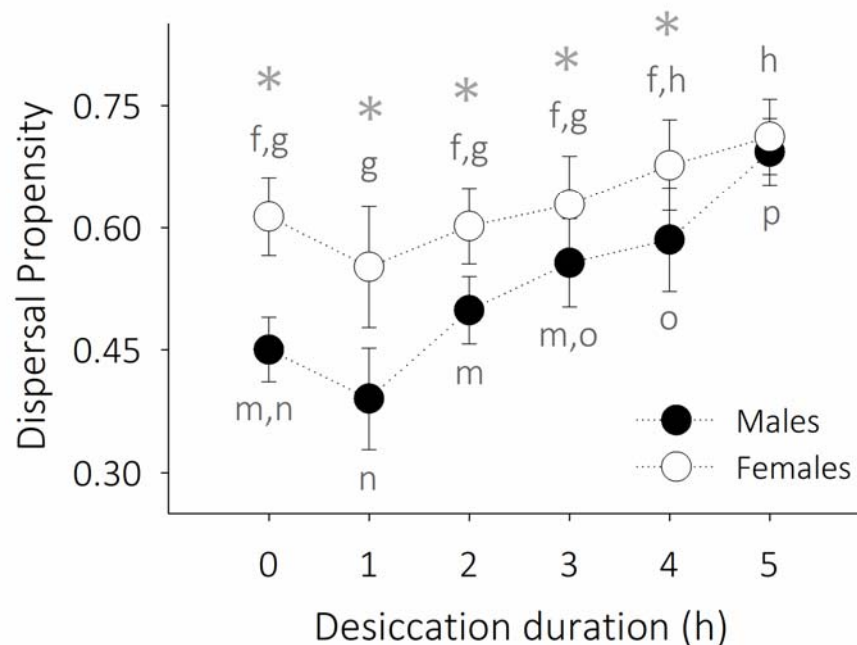306   desiccation leading to greater dispersal.

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Fig. 3: Effect of desiccation duration on dispersal propensity (Experiment 2). Dispersal propensity (± SE) for age-matched flies from an outbred baseline population (DB$_4$) subjected to desiccation stress for different durations (0–5 h). Each point represents the average of 10 replicates (each with 120 individuals). For a given sex, the changes in dispersal are examined by comparing the propensity means across the six desiccation durations (significant differences denoted using different lower-case letters: starting with m for males and f for females). Asterisks (*) denote a significant difference in male and female dispersal for a given desiccation duration. See Supplementary Text S2.2 for the exact p values.

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308   3.3 Desiccation stress as a sex-biased cost of dispersal

309   Next, we examined the role of desiccation stress as a cost of dispersal using four dispersal-

310   selected populations (VB$_{1-4}$) and their corresponding non-selected controls (VBC$_{1-4}$)

311   (Experiment 3). Desiccation resistance data from this experiment revealed a significant

312   *dispersal × sex* interaction ($\chi^2 = 9.52$, p = 0.002), with males experiencing a relatively larger

313   desiccation cost of dispersal (p < 10^{-4}, d = 1.86 (large)) (Fig. 4A, 4B) than females (p < 10^{-4},

314   d = 0.42 (small)) (Fig. 4C, 4D). Moreover, the *dispersal selection × dispersal* ($\chi^2 = 2.33$, p =

315   0.13) and *dispersal selection × dispersal × sex* ($\chi^2 = 0.19$, p = 0.66) interactions were not

316   significant, indicating that this result was consistent for both control (VBC) and dispersal-

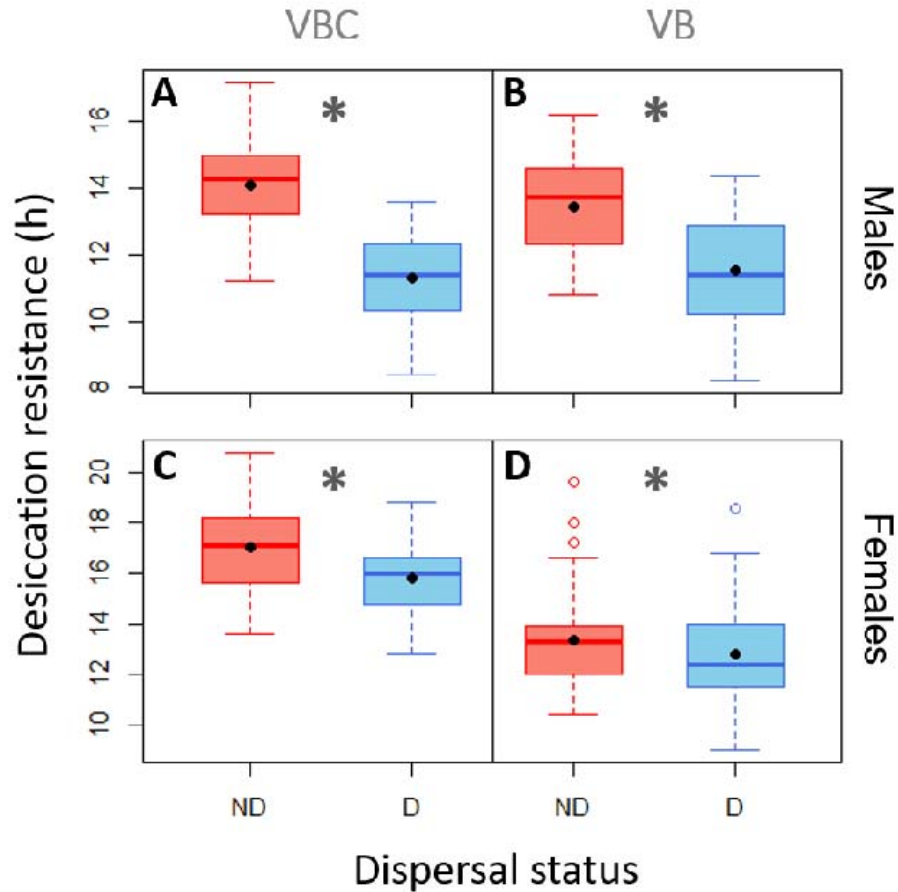317   selected (VB) populations (Supplementary Text S2.3).

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Fig. 4: Dispersal evolution and desiccation cost of dispersal (Experiment 3). Desiccation resistance of non-dispersers (ND) and dispersers (D) from VB$_{1-4}$ (dispersal-selected) and VBC$_{1-4}$ (control) populations. Data for males and females are presented in the top and bottom rows, respectively. Edges of the boxplots represent 25th and 75th percentiles of the data. Asterisks (*) indicate a significant difference (p < 0.05) between ND and D flies within a given panel. See Supplementary Text S2.3 for the exact p values.

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319

320   3.4 Significant cost of dispersal for females in terms of fecundity

321   As minimal or no desiccation cost of dispersal was observed for females (Sections 3.1 and

322   3.3), we investigated if there was a reproductive cost of dispersal for the females (Experiment

323   4). Analysis of the female fecundity data from this experiment (presented in Supplementary

324   Text S2.4) revealed a significant *scenario* × *dispersal* interaction (χ^2 = 17.90, p = 0.0001).

325   Pairwise comparisons for this interaction revealed no significant difference between

326   dispersers and non-dispersers under the *Control* (p = 0.63) (Fig. 5B) and *Cause* (p = 0.16)

327   scenarios (Fig. 5A), but a significant difference in the *Cost* scenario: disperser females had a

328   lower fecundity than non-disperser females (p = 0.0001, d = 0.68 (medium)) (Fig. 5C).

329   Therefore, we concluded that female flies pay a cost of dispersal in terms of their fecundity.
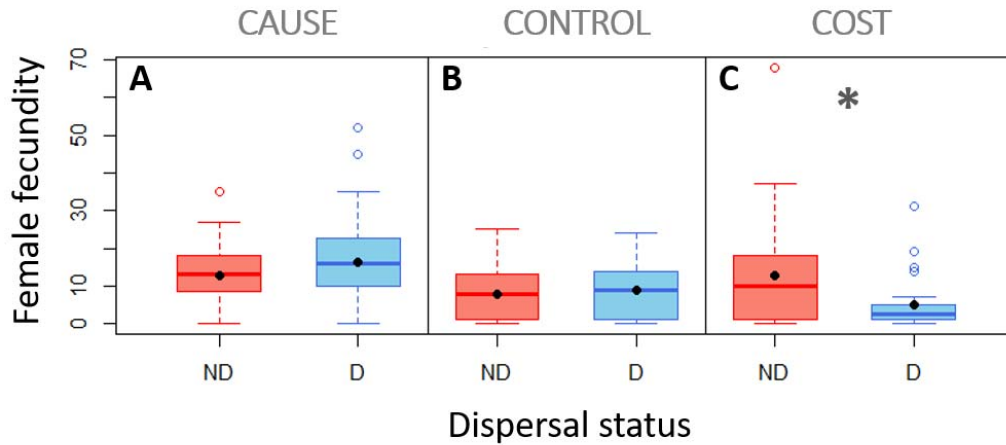

Fig. 5: Female fecundity as cause vs. cost of dispersal (Experiment 4). Female fecundity for non-disperser (ND) and disperser (D) flies from an outbred, baseline population (DB_4), under three scenarios: *Cause*, *Control*, and *Cost*. Edges of the boxplots represent 25th and 75th percentiles of the data. Asterisks (*) indicate a significant difference (p < 0.05) between ND and D flies within a given panel. See Supplementary Text S2.4 for the exact p values.

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4. Discussion

4.1 Desiccation stress as a cause of dispersal in both sexes

Environmental stress, among other things, can serve as a major cause of biological dispersal. At the same time, the very process of dispersal can be stressful to individuals. When monitored after a dispersal event, the stress-resistance ability of organisms are often found to be lower (Graves *et al.* 1992). This decrease can come about in three different ways. First, the dispersers might be the ones that were more susceptible to the stress, and hence they dispersed. Second, even if the stress resistance of the dispersers is inherently similar to that of the non-dispersers, the energy spent in the act of dispersal reduces the stress-resistance ability of the former. Third, it might be an interaction of the above two scenarios. Unfortunately, these questions are very difficult to answer, particularly when there is no *a priori* way of distinguishing between a disperser and a non-disperser. Here, we investigated this complex relationship, using desiccation as the type of stress and fruit flies as a model system. Our experimental design allowed us to explicitly control for other confounds when a particular aspect of the desiccation-dispersal relationship was being examined.

To begin with, Experiment 1 revealed that the disperser (D) flies had a lower desiccation resistance than the non-disperser (ND) flies under the *Cause* scenario (Figs. 2A and 2D). Comparing the results with the *Control* scenario, which showed no difference between ND and D flies (Figs. 2B and 2E), we could conclude that desiccation stress indeed served as a significant driver of dispersal for both male and female flies. This is in line with the expectation from literature that dispersal is one of the foremost ways for escaping unfavourable conditions (Gerber & Kokko 2018), not only in animal taxa (Cremer & Heinze 2003; Riotte-Lambert & Matthiopoulos 2020) but also in plants (Martorell & Martínez☐López 2014). While this is not a surprising result, our study demonstrates it

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356   explicitly using a unique setup, where we were able to control for the possible confound of

357   desiccation as a cost of dispersal (Fig. 1A).

358   Going a step further, we demonstrate in Experiment 2 how *Drosophila* dispersal changes with

359   increasing desiccation stress (Fig. 3). Given that desiccation resistance is highly correlated

360   with glycogen content in fruit flies (Gibbs, Chippindale & Rose 1997), one might have

361   expected a decrease in dispersal at longer desiccation durations, where the flies likely faced a

362   severe depletion of their glycogen reserves (Folk & Bradley 2004; Bazinet *et al.* 2010).

363   Surprisingly however, this was not the case in Experiment 2, where flies of both sexes

364   showed a nearly monotonic increase in their dispersal propensity with increasing desiccation

365   stress (Fig 3). This means that, at least for the duration of desiccation stress (up to 5 h)

366   imposed in Experiment 2, the flies were in a state to successfully initiate dispersal. However,

367   as a corollary, it also means that organisms likely do not disperse until the stress turns acute,

368   which may make them more susceptible to dispersal-related risks and costs (see Section 4.2).

369   It is possible that this delay in emigration could be a function of how long it takes to initiate

370   the stress physiological response. Overall, we speculate that the ability to perceive stress

371   would play a role in shaping the dispersal-mediated escape response from stressful habitats.

372   Since dispersal is also known to incur various costs (reviewed in Bonte *et al.* 2012), the

373   process of dispersal itself can induce stress or increase the susceptibility of dispersing

374   individuals to stress. We explored the potential desiccation cost of dispersal using the *Cost*

375   scenario, in Experiments 1 and 3.

376   4.2 Sex-biased cost of dispersal in terms of desiccation stress

377   Given that active dispersal involves expenditure of energy, it is likely that flies spend a part

378   of their glycogen reserves during dispersal (Graves *et al.* 1992), which can reduce their

379   desiccation resistance following a dispersal event. Experiment 1 confirmed a cost of dispersal

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380   in terms of their desiccation resistance, although it was not symmetric between the two sexes.

381   A significant desiccation cost of dispersal was observed for males (Fig. 2C) but not for

382   females (Fig. 2F) in the DB$_4$ population. Similarly, Experiment 3 revealed that the

383   desiccation cost of dispersal was much higher in males (Fig. 4A, 4B) than in females (Fig.

384   4C, 4D) (see section 3.3 for the exact effect sizes). As both dispersal-selected (VB) and non-

385   selected control (VBC) flies showed a male-biased desiccation cost, we concluded that the

386   evolution of dispersal did not alter the immediate desiccation cost of dispersal between these

387   populations.

388   A potential explanation for the sex bias in desiccation cost is the sexual dimorphism in body

389   size and desiccation resistance of *D. melanogaster* adults. A positive association between

390   desiccation resistance and body size is well documented in adult fruit flies (Parsons 1970;

391   Clark & Doane 1983). Given that female fruit flies are typically larger than their male

392   counterparts, they typically tend to have a higher desiccation resistance as well (Gibbs,

393   Chippindale & Rose 1997; Matzkin, Watts & Markow 2007; Mishra *et al.* 2018a). As a

394   result, the females likely had greater resources to begin with, which allowed them to

395   successfully undertake dispersal without paying a high desiccation cost. This is also

396   congruent with the observation that dispersal evolution has not led to a change in the body

397   size of VB females relative to their VBC controls (Mishra *et al.* 2018a; Tung *et al.* 2018a).

398   It is possible that the dispersal cost for females manifests not in terms of their somatic

399   maintenance (here, desiccation resistance), but instead their reproductive potential. This is in

400   line with the results of several life-history studies on trade-offs that show a reproductive cost

401   instead of somatic costs in females (Miyatake 1997; Ghalambor & Martin 2001; Djawdan *et*

402   *al.* 2004; Muller-Landau 2010). In such cases, female fecundity is often one of the first traits

403   to exhibit this cost. Given the energy-intensive nature of active dispersal (as evidenced by the

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404   dispersal cost borne by males in this study), female fecundity could show a cost of dispersal.

405   Therefore, we next investigated the association between female fecundity and dispersal.

406   4.3 Fecundity cost of dispersal for female flies

407   The relationship between dispersal and fecundity varies across taxa. A negative association

408   between dispersal and fecundity has been reported in several wing-dimorphic insects

409   (reviewed in Guerra 2011), wing-monomorphic insects (reviewed in Tigreros & Davidowitz

410   2019), as well as other taxa such as *C. elegans* (Friedenberg 2003). These results are typically

411   explained as a developmental or energetic cost of dispersal in terms of fecundity. In contrast,

412   a positive association between dispersal and fecundity has been observed in many

413   mammalian taxa (reviewed in Stevens *et al.* 2014). Here, the typical explanation is twofold.

414   First, individuals with better body condition, including higher fecundity, could be better able

415   to complete dispersal. Second, high fecundity could lead to high dispersal via increased kin

416   competition in a given habitat. Of course, it is also possible that the dispersal-fecundity

417   relationship, like other dispersal-trait associations, is modulated by the environmental context

418   (e.g. Legrand *et al.* 2016; Mishra *et al.* 2018a). For instance, the fecundity cost of dispersal

419   may be particularly strong under limiting resources. Similarly, the positive association

420   between dispersal and fecundity might be altered by the population density and level of

421   resources in the originating patch (e.g. Einum, Sundt☐Hansen & H. Nislow 2006). Therefore,

422   experiments under controlled conditions, which can take the ecological context into account,

423   can provide important insights into the relationship between fecundity and dispersal.

424   Experiment 4 revealed that, while there was no difference under the *Cause* and *Control*

425   scenarios, D females had a significantly lower fecundity than ND flies in the *Cost* scenario

426   (Fig. 5). What makes our result interesting is that females showed a fecundity cost before the

427   somatic cost of dispersal, at least in terms of desiccation resistance (*cf.* Figs. 2F and FC). A

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428   plausible explanation for this is that, under stressful conditions, individuals may prioritize

429   survival over potential reproduction. This has been observed in other life-history traits as

430   well, where allocation of resources into somatic maintenance can, at times, take priority over

431   reproductive investment (Djawdan *et al.* 2004; Muller-Landau 2010; Martorell &

432   Martínez□López 2014). In particular, given that dispersal is a key life-history trait (Bonte &

433   Dahirel 2017) with several potential costs (Bonte *et al.* 2012), the fecundity trade-off

434   observed here is in line with the observations for other wing-monomorphic insects (Tigreros

435   & Davidowitz 2019).

436

437   4.4. Implications

438   Our results revealed desiccation as a cause of dispersal for both sexes in *Drosophila*

439   *melanogaster*, and dispersal propensity of both male and female flies increased with

440   increasing desiccation duration. In addition, we observed a male-biased cost of dispersal in

441   terms of desiccation resistance, while the female flies paid a fecundity cost of dispersal. We

442   discuss some implications of our results below.

443   First, these results demonstrate that the relationship between stress and dispersal is likely

444   complicated. On one hand, stress is likely to drive dispersal of individuals away from an area.

445   On the other hand, dispersing individuals incur a further cost of dispersal in terms of

446   increased stress. Therefore, early dispersers from a population may be the least stress-tolerant

447   individuals. In contrast, highly stress-tolerant individuals could delay emigration in response

448   to a stress. As a result, if dispersal occurs across habitats with high connectivity, stress-

449   intolerant individuals may have the highest dispersal propensity (e.g. Fig. 3). However, if the

450   inter-habitat connectivity is poor, only the relatively stress-resistant individuals in a

451   population would be able to undertake dispersal successfully by surviving the large dispersal

452   costs.

453   Second, sex differences in the somatic costs of dispersal may effectively lead to instances of

454   sex-biased dispersal, even if a similar number of male and female individuals emigrate from a

455   given area. This is because the stress-sensitive sex (e.g. males in the current study) may not

456   be able to complete dispersal as successfully as the stress-resistant sex (here, females). As a

457   result, in the species where mating occurs after a dispersal event, such differences can lead to

458   a skew in the local sex ratio of the dispersed population and consequently mate limitation.

459   Moreover, the sex-biased nature of dispersal costs can result in demographic consequences

460   through dispersal syndromes (Mishra *et al.* 2018a; Shaw, Kokko & Neubert 2018). For

461   instance, if the fecundity of immigrant females in a new area is reduced as a consequence of

462   dispersal, then they may not be able to compete with the resident females in that area. As a

463   result, the apparent prioritization of fitness cost over somatic cost in females, as observed

464   here, can hamper their settlement ability in a new habitat.

465   Finally, while dispersal is often considered an effective escape route against environmental

466   stress (Boeye *et al.* 2013; Travis *et al.* 2013), it might not be enough to offset the fitness

467   reduction caused by changing climatic conditions (Buckley, Tewksbury & Deutsch 2013).

468   The situation might worsen further with dispersal-associated costs that hamper the stress-

469   handling ability of individuals and their biological fitness (Cheptou *et al.* 2008).

470   Consequently, there is a need to incorporate information on the physiological condition of

471   dispersers in models that consider dispersal as a mode of escape from stressful habitats.

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Acknowledgements

Authors' Contributions

AM and SD conceived the ideas and designed methodology; AM, ST, VRSS and PMS collected the data; AM analyzed the data; AM and SD led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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668

669   Supporting Information for

670   **Desiccation stress acts as cause as well as cost of dispersal in *Drosophila melanogaster***

671   **Authors:** Abhishek Mishra, Sudipta Tung, V.R. Shree Sruti, P.M. Shreenidhi and Sutirth Dey*

672   ***Name and address of the corresponding author:**

673   Sutirth Dey

674   Professor, Biology Division

675   Indian Institute of Science Education and Research, Pune

676   Dr. Homi Bhabha Road, Pune, Maharashtra, India 411 008

677   Tel: +91-20-25908054

678   Email: s.dey@iiserpune.ac.in

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680 **Text S1. Assay details**

681 <u>Text S1.1 Desiccation resistance assay (Experiments 1 and 3)</u>

682 Desiccation resistance for a fly was measured as the duration that it could survive without food and
683 moisture. To quantify this, same-sex groups of 10 flies each were introduced into empty transparent
684 vials and monitored until the death of the last fly in each vial, in a well-lit environment maintained at
685 25 °C. The survivorship checks were conducted every 2 hours, and 10 such replicate vials were used
686 per sex.

687

688 <u>Text S1.2 Dispersal assay (Experiment 2)</u>

689 For every two-patch dispersal setup (replicate), we counted the number of male and female flies
690 that reached the destination during each of the 15-min intervals until the end of dispersal assay (2
691 h). In addition, we recorded the number and sex of flies that emigrated from the *source* but did not
692 reach the *destination*, i.e. those found within the *path* tube at the end of the dispersal assay.

693 These data were used to estimate the dispersal propensity, i.e. the proportion of flies that initiated
694 dispersal from the source, as:

$$\text{Dispersal Propensity} = \frac{(\sum_i n_i) + n_p}{N}$$

695 where n_i is the number of flies that reached the destination during the ith 15-min interval, n_p is the
696 number of flies found within the path at the end of dispersal assay and N is the total number of flies
697 introduced in the setup (here, 120).

698

699 <u>Text S1.3 Fecundity assay (Experiment 4)</u>

700 Female fecundity was assessed as the number of eggs laid per female over a 12-h period. The flies
701 were anaesthetized under mild CO_2 and pairs of one male and one female each were introduced into
702 individual 50-mL centrifuge tubes containing a banana-jaggery food cup. The tube had provision for
703 aeration and the food in the food cup provided a surface for laying eggs. Forty such replicates were
704 set up per group (i.e. dispersers/non-dispersers) per scenario. The setups were left undisturbed for
705 12 hours in a well-lit environment maintained at 25 °C. At the end of 12 hours, the flies were
706 discarded, and the eggs laid on the food were counted under a stereo microscope.

707

708 **Text S2. Detailed statistical analyses**

709 <u>Text S2.1 Experiment 1</u>

```
710  library(lme4)
711  m_Exp1 <- lmer(Desi_res ~ Scenario*Dispersal*Sex +
712  (1|Scenario:Dispersal:Sex:Vial), data = d_Exp1)
713
714  library(car)
715  Anova(m_Exp1, type = 'III')
```

```
Analysis of Deviance Table (Type III Wald chisquare tests)

Response: Desi_res
                           Chisq Df Pr(>Chisq)
(Intercept)            2761.3559  1  < 2.2e-16 ***
Scenario                 59.1629  2  1.422e-13 ***
Dispersal                 8.0429  1   0.004568 **
Sex                     162.1124  1  < 2.2e-16 ***
Scenario:Dispersal        3.8236  2   0.147815
Scenario:Sex              4.3361  2   0.114399
Dispersal:Sex             0.0087  1   0.925823
Scenario:Dispersal:Sex    7.1993  2   0.027333 *
```

716

```
717  library(emmeans)
718  em_Exp1 <- emmeans(m_Exp1, ~Scenario:Dispersal:Sex)
719  pairs(em_Exp1, simple = "Dispersal")
```

```
Scenario = Cause, Sex = F:
 contrast estimate    SE  df t.ratio p.value
 Si - So    -1.698 0.599 107 -2.836  0.0055


Scenario = Control, Sex = F:
 contrast estimate    SE  df t.ratio p.value
 Si - So    -0.560 0.583 107 -0.961  0.3387


Scenario = Cost, Sex = F:
 contrast estimate    SE  df t.ratio p.value
 Si - So    -0.107 0.584 108 -0.182  0.8556


Scenario = Cause, Sex = M:
 contrast estimate    SE  df t.ratio p.value
 Si - So    -1.620 0.583 107 -2.780  0.0064


Scenario = Control, Sex = M:
 contrast estimate    SE  df t.ratio p.value
 Si - So    -0.820 0.583 107 -1.407  0.1622


Scenario = Cost, Sex = M:
 contrast estimate    SE  df t.ratio p.value
 Si - So    -2.900 0.583 107 -4.977  <.0001


Degrees-of-freedom method: kenward-roger
```

720
721

32

722   Text S2.2 Experiment 2

```
723    library(lme4)
724    m_Exp2 <- glmer(cbind(Disp, Nondisp) ~ Desi_duration*Sex + (1|Day), data =
725    d_Exp2, family = binomial)

726
727    library(car)
728    Anova(m_Exp2, type = 'III')
```

```
       Analysis of Deviance Table (Type III Wald chisquare tests)

       Response: cbind(Disp, Nondisp)
                          Chisq Df Pr(>Chisq)
       (Intercept)       9.2976  1   0.002294 **
       Desi_duration    42.5907  5   4.473e-08 ***
       Sex              33.2183  1   8.237e-09 ***
       Desi_duration:Sex 17.1023  5   0.004310 **
```

729

```
730    library(emmeans)
731    em_Exp2 <- emmeans(m_Exp2, ~Desi_duration:Sex)
732    pairs(em_Exp2, simple = "Desi_duration")
```

```
       Sex = Female:
        contrast estimate    SE  df z.ratio p.value
        0 - 1      0.2645 0.120 Inf   2.209 0.2334
        0 - 2      0.0509 0.121 Inf   0.422 0.9983
        0 - 3     -0.0663 0.121 Inf  -0.546 0.9942
        0 - 4     -0.2879 0.123 Inf  -2.334 0.1802
        0 - 5     -0.4588 0.125 Inf  -3.660 0.0034
        1 - 2     -0.2136 0.119 Inf  -1.788 0.4732
        1 - 3     -0.3308 0.120 Inf  -2.752 0.0654
        1 - 4     -0.5524 0.122 Inf  -4.521 0.0001
        1 - 5     -0.7233 0.124 Inf  -5.822 <.0001
        2 - 3     -0.1172 0.121 Inf  -0.968 0.9281
        2 - 4     -0.3388 0.123 Inf  -2.754 0.0652
        2 - 5     -0.5097 0.125 Inf  -4.075 0.0007
        3 - 4     -0.2216 0.124 Inf  -1.791 0.4718
        3 - 5     -0.3925 0.126 Inf  -3.120 0.0223
        4 - 5     -0.1709 0.128 Inf  -1.339 0.7635

       Sex = Male:
        contrast estimate    SE  df z.ratio p.value
        0 - 1      0.2574 0.120 Inf   2.150 0.2618
        0 - 2     -0.2025 0.118 Inf  -1.712 0.5235
        0 - 3     -0.4471 0.119 Inf  -3.768 0.0023
        0 - 4     -0.5677 0.119 Inf  -4.765 <.0001
        0 - 5     -1.0584 0.123 Inf  -8.593 <.0001
        1 - 2     -0.4599 0.119 Inf  -3.850 0.0016
        1 - 3     -0.7044 0.120 Inf  -5.878 <.0001
        1 - 4     -0.8251 0.120 Inf  -6.858 <.0001
        1 - 5     -1.3158 0.124 Inf -10.582 <.0001
        2 - 3     -0.2445 0.118 Inf  -2.066 0.3051
        2 - 4     -0.3651 0.119 Inf  -3.073 0.0258
        2 - 5     -0.8559 0.123 Inf  -6.967 <.0001
        3 - 4     -0.1206 0.119 Inf  -1.012 0.9140
        3 - 5     -0.6114 0.123 Inf  -4.965 <.0001
        4 - 5     -0.4908 0.124 Inf  -3.971 0.0010

       Results are given on the log odds ratio (not the response) scale.
       P value adjustment: tukey method for comparing a family of 6 estimates
```

733

734

735   `pairs(em_Exp2, simple = "Sex")`

```
Desi_duration = 0:
 contrast       estimate    SE  df z.ratio p.value
 Female - Male    0.6905 0.120 Inf 5.764   <.0001

Desi_duration = 1:
 contrast       estimate    SE  df z.ratio p.value
 Female - Male    0.6834 0.120 Inf 5.705   <.0001

Desi_duration = 2:
 contrast       estimate    SE  df z.ratio p.value
 Female - Male    0.4370 0.119 Inf 3.667   0.0002

Desi_duration = 3:
 contrast       estimate    SE  df z.ratio p.value
 Female - Male    0.3097 0.120 Inf 2.575   0.0100

Desi_duration = 4:
 contrast       estimate    SE  df z.ratio p.value
 Female - Male    0.4107 0.123 Inf 3.348   0.0008

Desi_duration = 5:
 contrast       estimate    SE  df z.ratio p.value
 Female - Male    0.0908 0.129 Inf 0.707   0.4797

Results are given on the log odds ratio (not the response) scale.
```

736

737

738   Text S2.3 Experiment 3

```
739  library(lme4)
740  m_Exp3 <- lmer(Desi_res ~ Sel*Dispersal*Sex + (1|Block) +
741  (1|Sel:Dispersal:Sex:Block:Vial), data = d_Exp3)

742  library(car)
743  Anova(m_Exp3, type = 'III')

     Analysis of Deviance Table (Type III Wald chisquare
     tests)

     Response: Desi_res
                       Chisq Df Pr(>Chisq)
     (Intercept)      499.5846  1  < 2.2e-16 ***
     Sel               90.7786  1  < 2.2e-16 ***
     Dispersal          3.5418  1   0.059840 .
     Sex               17.1953  1  3.373e-05 ***
     Sel:Dispersal      2.3279  1   0.127075
     Sel:Sex           51.2349  1  8.195e-13 ***
     Dispersal:Sex      9.5162  1   0.002037 **
     Sel:Dispersal:Sex  0.1944  1   0.659241
744

745  library(emmeans)
746  em_Exp3 <- emmeans(m_Exp3, ~Dispersal:Sex)
747  pairs(em_Exp3, simple = "Dispersal")

     Sex = F:
      contrast estimate    SE  df z.ratio p.value
      Si - So    -0.928 0.222 Inf  -4.187 <.0001

     Sex = M:
      contrast estimate    SE  df z.ratio p.value
      Si - So    -2.445 0.225 Inf -10.879 <.0001

     Results are averaged over the levels of: Sel
     Degrees-of-freedom method: asymptotic
748

749
```

750   Text S2.4 Experiment 4

```
751     library(stats)
752     m_Exp4 <- glm(Fecundity ~ Scenario*Dispersal, data = d_Exp4, family =
753     quasipoisson)

754     library(car)
755     Anova(m_Exp4, type = 'III')

        Analysis of Deviance Table (Type III tests)

        Response: Fecundity
                            LR Chisq Df Pr(>Chisq)
        Scenario              31.679  2  1.321e-07 ***
        Dispersal              2.004  1  0.1568845
        Scenario:Dispersal    17.897  2  0.0001299 ***
756

757     library(emmeans)
758     em_Exp4 <- emmeans(m_Exp4, ~Scenario:Dispersal)
759     pairs(em_Exp4, simple = "Dispersal")

        Scenario = Cause:
         contrast estimate    SE  df z.ratio p.value
         Si - So     0.243 0.172 Inf  1.410  0.1584

        Scenario = Control:
         contrast estimate    SE  df z.ratio p.value
         Si - So     0.109 0.227 Inf  0.478  0.6324

        Scenario = Cost:
         contrast estimate    SE  df z.ratio p.value
         Si - So    -0.944 0.246 Inf -3.844  0.0001

        Results are given on the log (not the response) scale.
760

761
```