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Higher flight activity in the offspring of migrants compared to residents in a migratory insect

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1   **Higher flight activity in the offspring of migrants compared to residents in a migratory**

2   **insect**

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17

18   **Abstract**

19

20   Migration has evolved among many animal taxa and migratory species are found across all

21   major lineages. Insects are the most abundant and diverse terrestrial migrants, with trillions of

22   animals migrating annually. Partial migration, where populations consist of resident and

23   migratory individuals, is ubiquitous among many taxa. However, the underlying mechanisms

24   are relatively poorly understood and may be driven by physiological, behavioural or genetic

25   variation within populations. We investigated the differences in migratory tendency between

26   migratory and resident phenotypes of the hoverfly, *Episyrphus balteatus*, using tethered flight

27   mills. Further, to test whether migratory flight behaviour is heritable, we compared the flight

28   behaviour of laboratory-reared offspring of migrating, overwintering and summer animals.

29   Offspring of migrants attempted more flights than resident individuals. Interestingly, there

30   were no differences among wild-caught phenotypes with regard to number of flights or total

31   flight duration. Low activity in field-collected migrants might be explained by an energy

32   conserving state that migrants enter in to when under laboratory conditions. Our results

33   strongly suggest that flight behaviour is heritable and that genetic factors influence migratory

34   tendency in *E. balteatus*. These findings support the growing evidence that genetic factors

35   might play a role in partial migration and warrants careful further investigation.

36

39   **1. Introduction**

40

41   Migration has evolved independently among many animal taxa, and migrating animals

42   comprise a large proportion of all major lineages [1-4], with insects being the most abundant

43   and speciose terrestrial migrants [5-7]. The most common type of migration is partial

44   migration, which is defined by variation in migratory tendency within species [8-10]. Hence,

45   partially migratory populations are composed of a mixture of resident and migratory

46   individuals simultaneously [8,9]. Many examples of partially migratory species have been

47   reported in mammals, birds, fish and invertebrates [8].

48

49   As natural selection acts upon individuals, it is important to determine the underlying

50   mechanisms driving differences in individual migratory tendency [8]. Individual differences

51   in migratory tendency between animals of the same population may underpin the extent of

52   partial migration observed within species [8,11]. Possible drivers of differences in migratory

53   tendency can be heterogeneity within populations, such as physiological, morphological,

54   behavioural or genetic variation [8]. For example, in birds, morphological variation has been

55   shown to influence migratory tendency, with a smaller body size usually promoting migratory

56   behaviour [12]. Furthermore, behavioural differences driving migratory tendency have been

57   found in the fish *Rutilus rutilus*, where bold individuals are more likely to migrate [11], and in

58   insects [13]. This provides evidence for a strong behavioural component influencing partial

59   migration and suggests that differences in activity between individuals might influence

60   migratory tendency or variation in dispersal ability in insects [14].

61

62   Migratory behaviour has been shown to be heritable in a number of animal taxa. The

63   blackcap, *Sylvia atricapilla*, is a particularly well studied example, where migratory activity

64   and the behaviour associated with it, known as migratory restlessness, is strongly heritable

3

65   [e.g. 15,16]. The heritability of flight behaviour is also known in insects. For example,

66   migratory tendency has been shown to be heritable in the moths *Spodoptera exempta*,

67   *Mythimna separata*, *Helicoverpa armigera*, and the grasshopper *Melanoplus sanguinipes* [17-

68   20]. However, in other species such as *Locusta migratoria* and *Schistocerca gregaria*,

69   migratory behaviour is strongly influenced by environmental factors [19]. Therefore, we may

70   predict that behavioural differences and the heritability of behavioural traits may play an

71   important role in driving levels of partial migration within populations.

72

73   In Europe, some hoverfly species (Diptera, Syrphidae) are partially migratory, where part of

74   the population overwinters in the breeding grounds as adults, while others travel large

75   distances in search of a milder climate [13,21,22]. *Episyrphus balteatus* is the most common

76   migrant hoverfly in Europe and during winter a part of the population remains in the habitat

77   and overwinters as larvae, pupae or adults [23-26], whereas other individuals of the

78   population migrate south to the Mediterranean in autumn, where they breed throughout the

79   winter [27,28]. *Episyrphus balteatus* is an important pollinator and the larvae are efficient

80   aphid predators, playing a significant role in the biocontrol of agricultural crop pests [29-31].

81   Adult overwintering hoverflies are almost exclusively females that are in a facultative

82   reproductive diapause, whereas males of *E. balteatus* are thought to be unable to increase

83   their fat bodies and therefore are more susceptible to cold temperatures and are not expected

84   to overwinter [32,33]. Most studies so far have focused on the southward flights to the

85   Mediterranean in autumn [22,27,34]. Females migrate with an undeveloped reproductive

86   system, but sperm storage organs already full of sperm [33]. Currently, there is no description

87   of the northward flight back to central and northern Europe in spring [28,35]. The short life-

88   span of this species indicates that the migration system of *E. balteatus* is multi-generational,

89   with a single generation moving south in the autumn and successive generations moving north

90   in the spring [13,28,35], as is typical of many latitudinal insect migrations [5]. Interestingly,

4

91   no genetic differentiation has yet been found between different overwintering strategies of *E.*

92   *balteatus* [36], with very low genetic distances between populations and a lack of population

93   subdivision [37]. Therefore, it is thought all individuals may have the genetic material for the

94   expression of the different overwintering phenotypes and that their decision for one of the

95   strategies may depend on environmental and individual factors [36]. To date it is unclear

96   whether environmental or genetic factors are responsible for the decision of individuals to

97   migrate, or whether it is a combination of both.

98

99   Behavioural traits, such as the propensity to engage in long-distance flight and flight tendency

100   are crucial proxies for migratory potential or individual migratory tendency, and can be

101   quantified using tethered flight mills under controlled conditions [14]. Tethered flight trials

102   are a good way to measure flight behaviour and have been used to investigate flight potential

103   in a number of insect species [e.g. 14,18,38-41]. In this study, we investigated the migratory

104   tendency between different migratory phenotypes of *E. balteatus* using tethered flight mills.

105   In order to disentangle environmental effects that possibly trigger migration, such as weather

106   and food resources, we investigated differences in the flight behaviour of first generation

107   hoverflies deriving from overwintering, migrating and summer populations. Furthermore, to

108   investigate the importance of environmental effects, wild caught hoverflies from both

109   migrating and overwintering populations were also tested. Specifically, we aimed to answer

110   the following questions: (i) Do first generation hoverflies deriving from summer populations,

111   migrating or overwintering hoverflies differ in their flight behaviour? (ii) Do female and male

112   hoverflies descending from the different phenotypes differ in their flight behaviour? (iii) Do

113   female and male hoverflies captured during migration differ in their flight behaviour?

114

115   We expect individuals deriving from migrating populations to attempt more flights and spend

116   more time flying on the tethered flight mills than individuals deriving from overwintering or

117   summer populations. Moreover, we expect migrating and overwintering individuals collected

118   in the field to differ in their flight behaviour; with migrating animals showing a stronger

119   tendency to fly and a longer duration of flight. Since females have been observed in

120   significantly larger numbers while migrating, they are expected to attempt more flights and

121   spend more time flying on the mill than males.

122

123   **2. Methods**

124

125   **(a) Study animals**

126

127   Hoverflies for flight mill experiments were divided into three phenotypes: Resident

128   overwintering, resident summer and migratory. Resident overwintering and summer

129   hoverflies were caught in the surroundings of Bern, Switzerland (46°56'38"N, 7°26'49"E)

130   from April to November 2016 on sunny and warm days (figure S1). Individuals caught in

131   April were assigned to the overwintering phenotype, since only females were found during

132   this period of time. Males would indicate the possible return of migrants, as they do not

133   normally overwinter [33]. Additional overwintering flies were caught in November. Because

134   of harsh conditions in the beginning of November, migrants are believed to have started

135   migrating south already. Flies designated as summer individuals were caught in June and July,

136   in the same locations as overwintering flies. Migrating flies were captured at Col de Bretolet

137   (46°08'34.1"N 6°47'45.2"E), an alpine pass at 1923 m a.s.l. on the border between

138   Switzerland and France, in September and October 2016 (figure S1). Migrating flies were

139   caught during active migration, heading southwest over the pass in large numbers.

140

141   Hoverflies were put into flight cages (45 x 45 x 90 cm) that consisted of a white plastic frame

142   covered by nylon gauze in a climate chamber at 20ºC, with a day-night cycle of 16:8 h

143   light:dark. Flies were kept in groups of up to 20 individuals per cage. Each cage had a layer of

144   kitchen paper on the ground. One petri dish with moist cotton wool served as water supply

145   and an additional petri dish contained cotton wool with sugar water (10% sugar) and some

146   mashed pollen. Additionally, flies were provided with a small ball of pollen, powdered sugar

147   and honey (60% pollen, 30% powder sugar and 10% honey). All petri dishes were checked

148   and refilled daily.

149

150   **(b) Rearing of *E. balteatus* in the laboratory**

151

152   Hoverflies were reared in a climate chamber at 20ºC with a day-night cycle of 16:8 h light:

153   dark. A bean plant (*Vicia faba*) infested with aphids (*Acyrthosiphon pisum*) was placed into

154   the cage on the same day the hoverflies were caught, to stimulate egg laying. Bean plants

155   were checked for eggs and larvae daily. Larvae were placed individually in vials with a bean

156   leaf for shelter. Larvae were fed between 20-30 aphids daily. When pupated, the leaf and the

157   leftover aphids were taken out of the vial to increase the chance of a smooth emergence

158   [24,42]. Once emerged, the flies were put into a flight cage (45 x 45 x 90 cm) for between 12

159   and 24 hours, where they were given the possibility to feed and fly before the start of the

160   experiments.

161

162   **(c) Tethered flight mill experiments**

163

164   Tethered flight mills were used to investigate the flight behaviour of individual *E. balteatus*

165   (figure 1). Flight mills were designed at Rothamsted Research (Patent: [43]) and consist of a

166   lightweight wire arm suspended between two magnets, which results in almost no resistance

167   against the turning of the arm. This means that even weak fliers are able to turn the mill and

168   fly rotationally in a horizontal plane (see also [14]). The hoverfly was glued to a pin, attached

169   to one end of the arm of the mill, using a contact adhesive. A striped disc attached to the axis

170   turns with the arm (figure 1). A light sensor detects the movement of the disc and records the

171   distance flown (m), time spent flying (s) and flight speed (m/s). This data is used to calculate

172   different measurements of distance, duration and speed of specific flights, e.g. the furthest

173   flight or the first flight. The system used has five channels, allowing five individual insects to

174   be flown simultaneously. Data for each individual was processed using a custom-written

175   script (K.S.L.) in Matlab (The MathWorks Inc. 2012, see also [14,40] for further details on

176   the flight mills). Representative outputs from the flight mills are presented in figure S2.

177

178   Flight experiments were conducted between May and November 2016. Flies with damaged or

179   missing wings, or that were no longer able to fly were not used for the experiments. Before

180   gluing the hoverfly to the pin, a flight test was conducted by using a vial and releasing the fly

181   in to the air. If a hoverfly was not able to fly, it was excluded from any further experiments.

182   All individuals were randomly assigned to one of the 5 flight mills. Hoverflies were flown in

183   a clockwise rotation. Immediately after putting the flies on the flight mills, they were given a

184   piece of paper (approximately 1 x 1 cm) as a platform. At the start of the experiments, the

185   flight mill recording program was started and the piece of paper was removed as

186   simultaneously as possible from all flies. Hoverflies that did not start flying were stimulated

187   to fly once by putting a finger under their body and then removing it. During the experiments,

188   the flies were able to see each other, therefore, experiments were only conducted when at least

189   two individuals were present. All individuals were left on the flight mill for 4 hours,

190   regardless of their flight behaviour. Any hoverfly that looked damaged, unhealthy or had died

191   during the experiment was excluded from further analysis (5 of 232 animals tested).

192   Observational experiments were also carried out to distinguish flight from hovering: hovers

193   never resulted in the striped disc of the flight mill moving for more than 10 seconds, and thus

194   flights of less than 10 sec were excluded. Immediately after the flight mill experiments,

195   hoverflies were stored separately in a freezer (-25 ºC).

196

197   To investigate differences in flight ability and behaviour between wild caught individuals, a

198   subset of migratory individuals and all overwintering individuals captured in November were

199   used for tethered flight mill experiments directly. In this case, flies were given a minimum of

200   12 hours to acclimatize to the conditions in the climate chambers before the start of the

201   experiments. The experiment was started within the first 72 hours after capture. All

202   laboratory-reared individuals were flown within the first day after eclosion to ensure that they

203   did not already show reproductive behaviour [17].

204

205   **(d) Statistical Analysis**

206

207   All statistical analyses were conducted using R version 3.3.2 [44]. Initially, a generalised

208   linear model with a binomial error distribution was used to compare flight ability between the

209   first generation offspring of the different overwintering phenotypes. Sex and the interaction

210   between sex and phenotype were incorporated as fixed effects in the model. The same

211   procedure was applied to compare flight ability between wild caught overwintering and

212   migrating flies and for comparing the two treatments (wild caught and lab reared) within sex

213   and within phenotype (see Supplementary Material).

214

215   For further analysis, only data from flies that had flown at least two flights over 1 m/s were

216   included to ensure that we only analysed individuals that were able to fly on the mill. A

217   correlation test was performed to determine the relationship between the variables recorded

218   with the flight mills (figure S3). Three parameters considered important for migration were

219   initially chosen, total duration of flights, total distance flown and number of flights. As total

9

220   distance and total duration were correlated, further analyses were conducted using total

221   duration and number of flights. These two variables were log-transformed, so that the

222   residuals of the models visually indicated a normal distribution.

223

224   Total flight duration and number of flights were compared between first generation offspring

225   from the different overwintering phenotypes, using a binomial linear model. Sex and the

226   interaction between sex and phenotype were included as fixed effects in the model, since there

227   is expected to be a difference in flight behaviour between sexes. The same procedure was

228   done for the comparison of the different wild caught phenotypes. p-values were extracted

229   from the models by re-levelling of the intercept. Furthermore, male and female hoverflies

230   within the migratory phenotype were compared to each other using a linear model with sex as

231   a fixed effect.

232

233   **3. Results**

234

235   Overall, 72% of all individuals (168 of 232) were able to fly on the flight mills. There were no

236   significant differences in flight ability between the different phenotypes of the lab reared

237   animals, and between the two phenotypes collected in the field. Flight ability within

238   phenotype and within sex between wild caught and lab reared individuals was also not

239   significantly different (all $p \geq 0.353$, see Supplementary Material).

240

241   In both sexes, there was a trend for offspring of migrants (females: $n = 13$, males: $n = 13$) to

242   initiate more flights than offspring of overwintering females (females: $n = 23$, $p = 0.0497$;

243   males: $n = 23$, $p = 0.099$) and offspring of summer females (females: $n = 31$, $p = 0.026$;

244   males: $n = 31$, $p = 0.045$) (table 1, figure 2a). By contrast, the number of flights did not differ

245   between offspring of overwintering and summer females ($p > 0.05$ for males and females),

10

246   nor did it differ between the sexes in any of the phenotypes ($p > 0.05$ in all cases; table 1,

247   figure 2*a*).

248

249   Although the offspring of migrants initiated more flights than the offspring of the other

250   phenotypes, this increase did not typically translate into longer total flight duration. Only in

251   the case of male offspring from migrants compared with male offspring from summer flies

252   was there a significant increase in flight duration ($p = 0.044$; table 1, figure 2*b*). All other

253   pair-wise comparisons of phenotypes were non-significant, nor did flight duration differ

254   between the sexes in any of the phenotypes ($p > 0.05$ in all cases; table 1, figure 2*a*).

255

256   The trend for increased flight activity observed in the offspring of migrants compared to other

257   phenotypes was not supported by the comparison of wild-caught migrants (females: $n = 22$,

258   males: $n = 6$) and overwintering flies (females: $n = 8$, males: $n = 14$). In both sexes, number of

259   flights (figure 3*a*) and total flight duration (figure 3*b*) were not significantly different from

260   each other ($p > 0.05$ in all cases; table 2). In migrants, males had significantly longer total

261   flight durations than females ($p = 0.041$; figure 3*b*), but sexes did not differ in their flight

262   activity in the wintering flies. Comparison between wild caught and lab reared hoverflies

263   within phenotype and within sex showed no significant differences in the total number of

264   flights, nor total flight duration (table 3).

265

266   **4. Discussion**

267

268   We have demonstrated behavioural differences between the offspring of resident and

269   migrating *E. balteatus*, with the offspring of migrants undertaking more flights than the

270   offspring of summer or overwintering animals. Interestingly, we found no difference in flight

271   behaviour between actively migrating and overwintering hoverflies collected from the field,

11

272   under laboratory conditions. However, actively migrating males differed significantly in the

273   total duration of flights from migrating females, whereas there was no difference between the

274   sexes of migrant offspring. There were no differences observed in the flight ability on the mill

275   between sexes or phenotypes.

276

277   We observed behavioural differences between the offspring of hoverflies from different

278   overwintering strategies, suggesting that the decision to migrate in hoverflies might be

279   heritable. While Raymond *et al.* [36] did not detect any genetic differentiation between the

280   different overwintering strategies, using microsatellites, such differentiation might not be

281   detected using neutral markers [45]. First generation migrants initiated more flights

282   throughout the experiment than offspring of overwintering hoverflies. Heritability of

283   migratory tendency has been found in several species of insects, such as the moths *Mythimna*

284   *separata* [18] and *Spodoptera exempta* [20], and the grasshopper *Melanoplus sanguinipes*

285   [19], but this is the first investigation of this phenomenon in hoverflies. Interestingly, no

286   significant difference was detected in total flight duration between the offspring of migrating

287   and overwintering flies. It is believed that environmental cues such as wind [46-48] or a

288   change in temperature [49] are important for the departure or the continuation of migratory

289   movement in insects [5]. Hoverflies did not receive these environmental cues in the

290   laboratory, as temperature stayed constant throughout the experiment, and wind in the

291   laboratory was not possible, since flight mills record even the slightest movement. These

292   factors, or the absence of them, might be the reason why the short flights did not turn into

293   longer migratory flights. However, the increased number of attempts to fly in the offspring of

294   migrants suggests that they are more prone to flying than the offspring of other phenotypes.

295   Heritability in the urge to fly, also called migratory restlessness, is relatively well explored in

296   birds [e.g. 16,50-52]. Here, the number of attempted flights in *E. balteatus* could be

297   considered as a comparable assay to migratory restlessness, and may prove useful for

298   determining migratory propensity in hoverflies and other migratory insects.

299   No significant difference was detected between wild caught migrants and overwintering

300   individuals in number of flights or total flight duration. We believe that this relative lack of

301   activity is due to an energy conserving state these migrating animals fall into, when caught

302   out of active migration and transported to the laboratory. Similar results were found by

303   Odermatt *et al.* [13], who showed that migratory flies tended to be less active than summer

304   individuals, when tested in the laboratory. Migratory flights have been shown to involve

305   immense energy expenditures in birds [49] and this is also true for insects [53,54]. Thus, we

306   may expect physiological differences between migratory and resident phenotypes, as shown

307   by Attisano *et al.* [38] in milkweed bugs, *Oncopeltus fasciatus*. Another reason for the lack of

308   difference in flight behaviour between wild caught migratory and overwintering hoverflies

309   could be due to the absence of certain environmental cues in the laboratory. Similarly to

310   triggering migration, individuals collected from the field might depend on these

311   environmental cues for the continuation of their migratory flight. Since in the laboratory, they

312   are not exposed to these cues, they may conserve their energy for when they are able to

313   continue their migration [13].

314

315   Furthermore we found a difference in total flight duration between wild caught male and

316   female migrants, with male migrants undertaking significantly longer flights than females.

317   Male migrants do not have any reproductive constraints, whereas females migrate with an

318   undeveloped reproductive system [33]. During migration, females reallocate their energy

319   away from reproduction, but still experience reproductive constraints by maintaining sperm

320   storage organs [33]. This might explain the longer flight duration in males compared to

321   females. While no differences were shown in resting metabolic rate between male and female

322   *E. balteatus* [55], this is yet to be investigated in migrating individuals. No significant

13

323   difference in flight duration was observed between male and female offspring of migrants.

324   This might be explained by the offspring being freshly emerged and a maximum of 48 hours

325   old. Reproduction is not important in the first few days, and females only start laying eggs

326   after 12 days [56]. The number of flights was not significantly different between male and

327   female migrants and neither between male and female migrant offspring. However, this is

328   what we expect. Male and female hoverflies both migrate, although males are the less

329   abundant sex during migration [57]. Therefore, they were not expected to differ in their

330   propensity to fly. The number of flights is less restricted by the difference in energy

331   expenditure of the two sexes, since most of the energy is used during long flights.

332

333   In summary, we have shown that offspring of different phenotypes differ in their flight

334   behaviour, strongly suggesting genetic factors influencing migratory tendency in *E. balteatus*.

335   Moreover, we hypothesize that environmental cues do remain important for migration, as we

336   found no difference in flight behaviour between the different wild caught phenotypes.

337   Furthermore, we have shown behavioural differences between sexes within the migratory

338   phenotype, supporting the evidence for reproductive constraints of migrating females [33].

339   Future investigations should include studies under field conditions that are close to conditions

340   hoverflies experience during migration. Thereby, environmental factors could be included

341   into tethered flight mill experiments.

342

343   **Data accessibility.** Data are available from the Dryad Digital Repository

344   (http://dx.doi.org/10.5061/dryad.44hc2) [58].

345

346   **Author's contributions.** L.J.D., A.G., K.S.L., J.W.C., and M.H.M.M designed the

347   experiments. L.J.D. and A.G. carried out the experiments with assistance from K.S.L. and

348   M.H.M.M. L.J.D. wrote the initial draft of the manuscript with assistance from M.H.M.M,

349   and input and revisions from all authors.

350

351   **Competing interests.** The authors have no competing interests.

352

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355

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516

517   **Table 1.** Estimates from linear models comparing number of flights and total duration

518   between first generation females (F) and males (M) of different phenotypes.

519

Comparison	Estimate	SE	*P*
Number of flights (log)			
Migrating M vs. Overwintering M	-0.546	0.329	0.099
Migrating M vs. Summer M	-0.651	0.322	**0.045**
Overwintering M vs. Summer M	-0.105	0.271	0.700
Migrating F vs. Overwintering F	-0.652	0.329	**0.049**
Migrating F vs. Summer F	-0.759	0.337	**0.026**
Overwintering F vs. Summer F	-0.107	0.290	0.712
Migrating M vs. Migrating F	-0.308	0.372	0.408
Overwintering M vs. Overwintering F	-0.414	0.279	0.141
Summer M vs. Summer F	-0.417	0.282	0.142
Total duration (log)			
Migrating M vs. Overwintering M	-0.396	0.361	0.275
Migrating M vs. Summer M	-0.722	0.354	**0.044**
Overwintering M vs. Summer M	-0.326	0.298	0.277
Migrating F vs. Overwintering F	-0.201	0.361	0.580
Migrating F vs. Summer F	-0.265	0.371	0.477
Overwintering F vs. Summer F	-0.064	0.318	0.841
Migrating M vs. Migrating F	-0.412	0.409	0.315
Overwintering M vs. Overwintering F	-0.217	0.307	0.482
Summer M vs. Summer F	0.044	0.310	0.886

520

521   Significant differences ($P < 0.05$) are presented in bold. Transformations are presented in

522   parentheses.

523

22

524 **Table 2.** Estimates from linear models comparing number of flights and total duration

525 between females (F) and males (M) of different wild caught phenotypes.

526

Comparison	Estimate	SE	*P*
Number of flights (log)			
Migrating M vs. Overwintering M	-0.544	0.483	0.266
Migrating F vs. Overwintering F	0.146	0.408	0.722
Migrating M vs. Migrating F	-0.752	0.456	0.106
Overwintering M vs. Overwintering F	-0.062	0.438	0.887
Total duration (log)			
Migrating M vs. Overwintering M	-0.860	0.496	0.053
Migrating F vs. Overwintering F	0.191	0.419	0.650
Migrating M vs. Migrating F	-0.985	0.468	**0.041**
Overwintering M vs. Overwintering F	0.193	0.450	0.671

527

528 Significant differences ($P < 0.05$) are presented in bold. Transformations are presented in

529 parentheses.

530

531   **Table 3.** Estimates from linear models comparing number of flights and total duration

532   between wild caught and lab reared hoverflies within the same phenotype.

533

Comparison	Estimate	SE	*P*
Number of flights (log)			
Migrating M wild vs. Migrating M lab	-0.391	0.441	0.380
Migrating F wild vs. Migrating F lab	0.053	0.312	0.866
Overwintering M wild vs. Overwintering M lab	-0.393	0.363	0.282
Overwintering F wild vs. Overwintering F lab	-0.745	0.439	0.095
Total duration (log)			
Migrating M wild vs. Migrating M lab	-0.430	0.487	0.381
Migrating F wild vs. Migrating F lab	0.143	0.345	0.681
Overwintering M wild vs. Overwintering M lab	0.160	0.369	0.666
Overwintering F wild vs. Overwintering F lab	-0.249	0.447	0.579

534

535   Significant differences ($P < 0.05$) are presented in bold. Transformations are presented in

536   parentheses.

537

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24

539   **Figure captions**

540

541   **Figure 1.** Tethered flight mill. Labelled photograph of an individual flight mill (*a*) and a close

542   up of *Episyrphus balteatus* attached to the flight mill (*b*).

543

544   **Figure 2.** Number of flights (*a*) and flight duration in seconds (*b*) between phenotypes and

545   sexes of first generation hoverflies. For clarification, only significant p-values are indicated in

546   the graph. *$p < 0.05$. Number of flights and flight duration have been log transformed.

547

548   **Figure 3.** Number of flights (*a*) and total flight duration in seconds (*b*) between wild caught

549   migrating and overwintering hoverflies. For clarification, only significant p-values are

550   indicated in the graph. *$p < 0.05$. Number of flights and flight duration have been log
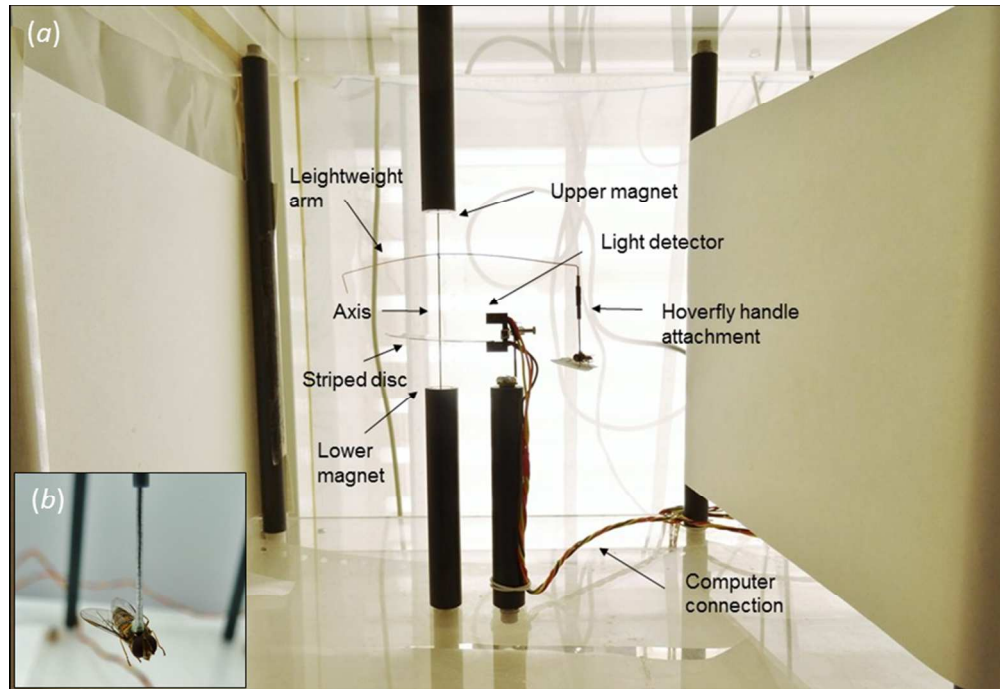
551   transformed.

Figure 1. Tethered flight mill. Labelled photograph of an individual flight mill (a) and a close up of Episyrphus balteatus attached to the flight mill (b).
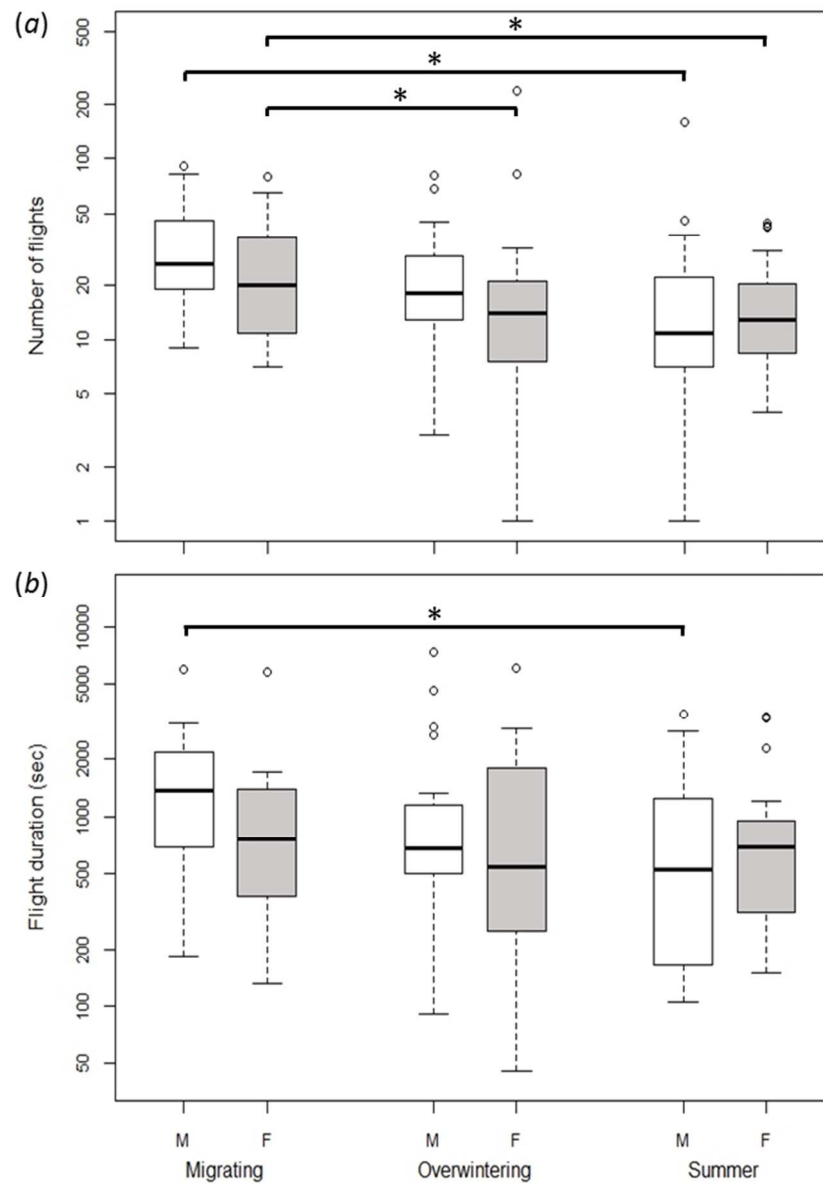
160x110mm (150 x 150 DPI)

Figure 2. Number of flights (a) and flight duration in seconds (b) between phenotypes and sexes of first generation hoverflies. For clarification, only significant p-values are indicated in the graph. *p < 0.05. Number of flights and flight duration have been log transformed.
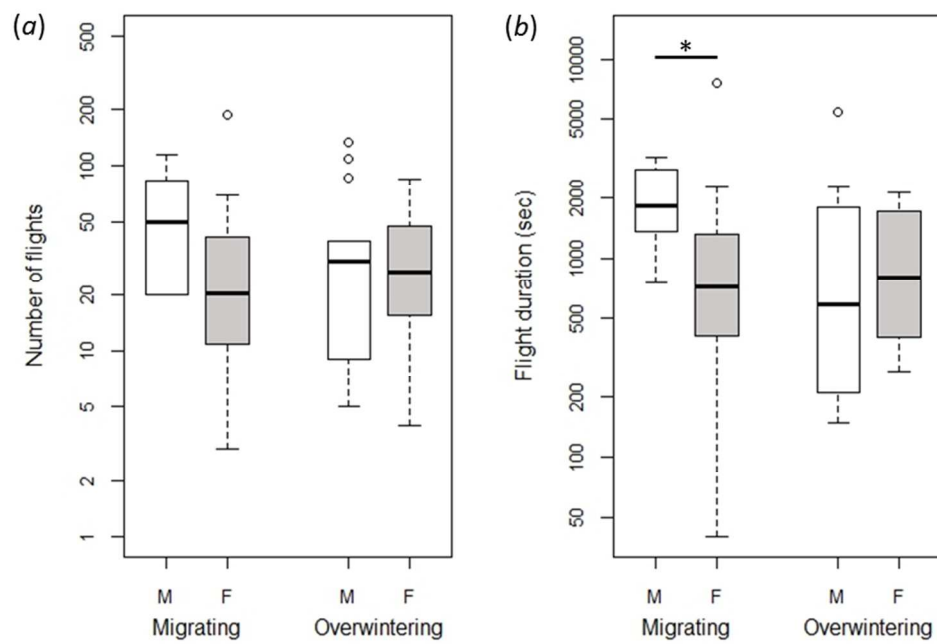
122x166mm (150 x 150 DPI)

Figure 3. Number of flights (a) and total flight duration in seconds (b) between wild caught migrating and overwintering hoverflies. For clarification, only significant p-values are indicated in the graph. *$p < 0.05$. Number of flights and flight duration have been log transformed.

163x109mm (150 x 150 DPI)