1   **Effects of floral symmetry and orientation on the consistency of pollinator's entry angle**

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3   Nina Jirgal[1,2*], Kazuharu Ohashi[2]

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5   [1]University of Manchester, Oxford Road, Manchester M13 9PL, United Kingdom

6   [2]Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572,

7   Japan

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9   *Author for correspondence (Tel: +44 7588361125, E-mail: ninajirgal@gmail.com)

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Abstract

Since the publication of Sprengel's (1793) observations, it has been considered that flowers with zygomorphic (or bilaterally symmetrical) corollas evolved to restrict the movement of pollinators into the flower by limiting the pollinator's direction of approach. However, little empirical support has been accumulated so far, except Culbert and Forrest (2016) who found that zygomorphy reduced variance in pollinator's flower entry angle. Our aim was to build on this work and observe whether floral symmetry or orientation had an effect on pollinator entry angle in a laboratory experiment using bumble bees, *Bombus ignitus*. Using nine different combinations of artificial flowers created from three symmetry types (radial, bilateral and disymmetrical) and three orientation types (upward, horizontal and downward), we tested the effects of these two floral aspects on the consistency of bee's entry angle. Our results show that horizontal orientation significantly reduced the variance in entry angle, while symmetry had little effect. We also found no significant interactions between angle and symmetry in their effect on entry angle. Thus, our results suggest that horizontal orientation forces the bees to orient themselves relative to gravity rather than the corolla and stabilizes their flower entry. This stabilizing effect may have been mistaken for the effect of zygomorphic corolla as it is presented horizontally in most species. Consequently, we suggest that the evolution of horizontal orientation preceded that of zygomorphy as indicated by some authors, and that the reason behind the evolution of zygomorphy should be revisited.

Keywords: approach consistency, artificial flowers, *Bombus*, floral orientation, floral symmetry, pollen placement

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51 **Declarations**

53 Availability of data: Data can be obtained from Nina Jirgal upon request.

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Introduction

76   Zygomorphy, or bilateral symmetry, in angiosperm flowers is suggested to have evolved independently in

77   multiple lineages from their ancestral radial form about 50MY after their initial emergence, which coincides

78   with the emergence of specialised pollinators (Citerne et al. 2010; Hileman 2014). Currently, 130 origins of

79   zygomorphy have been estimated, while only 69 reversions to actinomorphy, or radial symmetry, have occurred

80   (Reyes et al. 2016). Its emergence has been recognized as a key innovation as it is seen to be homoplastic in

81   extant angiosperms and is associated with species diversification (Woźniak and Sicard 2018). Indeed, radially

82   symmetric lineages comprise of fewer species than their bilaterally symmetric sister lineages (Sargent 2004;

83   Woźniak and Sicard 2018). Overall, zygomorphic flowers have a higher level of fitness compared to their

84   actinomorphic counterparts (Gómez et al. 2006).

85   Although there are many proposed hypotheses for the evolution of zygomorphy, they can be divided

86   into three major groups of from the perspective of benefits for the plant. The first one is that zygomorphy may

87   increase flower (re)visitation by pollinators through making the flowers easier to perceive, learn or forage.

88   Because zygomorphic flowers are morphologically more complex than non-zygomorphic ones, pollinators are

89   likely to be given more visual information on which to base their specific recognition of these flowers (Neal et

90   al. 1998). For example, zygomorphic flowers have a higher contour density, i.e., the dissected margin of

91   flowers, compared to actinomorphic ones (Anderson 1977; Dafni and Kevan 1997). Lehrer et al. (1985) have

92   shown that honeybees tend to scan the contour of flowers in a close range. This may suggest that zygomorphic

93   flowers exploit scanning behaviour of certain pollinators and help them to reach the floral resources (Dafni and

94   Kevan 1997). Alternatively, zygomorphic flowers often have elongated lower lips on which pollinators could

95   land easily (Sprengel 1793). The resultant decrease in landing time may lead to increased return visits by

96   experienced foragers (Neal et al. 1998).

97   The second group of hypotheses is that the complexity of the corolla restricts the type of pollinators

98   that could exploit the floral resources. Only a small subset of animals are able to reach the nectar in

99   zygomorphic flowers due to their complexity (Neal et al. 1998; Zhao et al. 2016; Krishna and Keasar 2018).

100   This would be advantageous if the complexity acts as a morphological filter that allows only effective

101   pollinators to extract nectar from the flower and contribute to its pollination (Krishna and Keasar 2018). In

102   addition, the filtering of pollinators according to their ability to handle flowers is suggested to increase

103   pollinator fidelity, as specialised pollinators mainly exploit fewer complex flower species rather than visiting

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104   many flower species with simple morphologies (Rogriguez-Girones and Santamaría 2016; Krishna and Kaesar

105   2018). This fidelity of pollinators would benefit flowers in terms of lowering heterospecific pollen transfer

106   (Krishna and Kaesar 2018).

107   The third hypothesis suggests that a zygomorphic corolla restricts the movement of the insect (Wang et

108   al. 2014; Ushimaru and Hyodo 2005; Neal et al. 1998), resulting in approaches that are more stable and

109   predictable in direction (Fenster et al. 2009). Zygomorphy has been linked to a gene that supresses the growth of

110   stamen (Rudall and Bateman 2004) and concentrates the reproductive parts of the flower in one location.

111   Indeed, O'Meara et al (2016) have shown that the diversification of zygomorphy is contingent on the presence

112   of a corolla and reduction of stamen. The concentration of stamens and stigmas to a narrower area, combined

113   with the predictable movement of the pollinator, would allow these reproductive parts to make more consistent

114   contact with the pollinator's body. This consistency would increase the conspecific pollen transfer while

115   decreasing the heterospecific pollen exchange with pollinator-sharing species (Muchhala and Thomson 2010;

116   Culbert and Forrest 2016).

117   Here we focus on the third hypothesis, which has often been assumed in studies of floral evolution

118   (e.g., Sargent 2004) but rarely tested empirically. The effect of corolla shape on pollinator entry angle has been

119   examined by Culbert and Forrest (2016) in a laboratory experiment using artificial flowers and bumble bees

120   (*Bombus impatiens*). Using circular (radially symmetric) and rectangular (disymmetric) flowers, they showed

121   that the approach consistency of bees was higher on disymmetric than on radial flowers. However, disymmetric

122   flowers are rare in nature and slightly different from zygomorphic (bilaterally symmetric) flowers. In addition,

123   all the artificial flowers in their experiment were oriented horizontally. While zygomorphic flowers are usually

124   seen oriented horizontally in nature, radial flowers are commonly oriented in an upward or downward manner.

125   Therefore, their results may not fully reflect the effect of corolla shape on entry angle consistency in natural

126   settings. Finally, Fenster et al. (2009) has suggested that the entry angle stabilisation observed in zygomorphic

127   flowers may be provided by the horizontal orientation in which they are presented, rather than the corolla

128   symmetry. Thus, it is possible that the effects of floral symmetry were intermingled with those of orientation in

129   the experiments by Culbert and Forrest (2016).

130   In this study, we aimed to evaluate both the effect of floral symmetry and orientation on the

131   consistency of pollinator's entry angle. As an extension of the experiment conducted by Culbert and Forrest

132   (2016), we have used artificial flowers with three symmetry types (actinomorphy, zygomorphy and

133   disymmetric) and oriented them in three ways (upward, horizontal and downward). By testing nine possible

134   combinations of symmetry and orientation, some of which rarely exist in nature, we tried to dissect and quantify

135   the effects of two floral features separately.

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137   **Materials and methods**

138   *Artificial flowers*

139   We used artificial flowers for the experiments, each consisting of a "corolla" cut from blue drawing paper

140   (~11.5 cm²), and a container for cotton, from which the sucrose solution ("nectar") can be collected by bees.

141   Each corolla shape represents one of the three types of floral symmetry: actinomorphy (circular; 38 mm

142   [diameter]), zygomorphy (triangular; 40 mm [base] x 57.5 mm [height]), and disymmetry (rectangular; 55 mm

143   [length] x 21 mm [width]). We used artificial flowers with identical appearance for the training and test phases,

144   the only difference being the configuration of the cotton container (see below).

145   A test flower had a 0.5-mL microcentrifuge tube as a container for a small piece of cotton. This

146   microcentrifuge tube was embedded into a larger (1.5-mL) microcentrifuge tube using white, odourless clay.

147   The opening of the smaller tube was at the same level as that of the larger tube. The cotton was inserted into the

148   smaller tube, and the nectar was added on top of it so that bees could access it easily. The cotton was used as a

149   fluid reservoir to prevent the nectar from leaking out of the microcentrifuge tube when the flower is not

150   upwardly presented, while allowing bees to easily access it. On the other hand, a training flower used a 1.5-mL

151   microcentrifuge tube as the container, into which a dental cotton roll was inserted to prevent the nectar from

152   leaking out. We chose a larger nectar reservoir for the training flower, so that the nectar would not run out

153   quickly and the bees would not lose their motivation. We also used six plastic petri dishes as supplementary

154   feeders during the training phase (two petri dishes per flower shape). Each petri dish had a hole cut into the

155   middle and a dental cotton was inserted. It absorbed the nectar in the petri dishes and allowed bees to access the

156   nectar.

157   Twelve flowers were positioned on a grid in a three-by-four pattern, with a 10-cm interval between the

158   centres of the adjacent flowers. Each was placed in either of the three positions: floor (grid lying flat on the floor

159   of the cage), wall (grid leaning against the back side of the cage at 90° to the ground), and ceiling (grid hung

160   upside down from the ceiling with metal hooks). Hereafter, we refer to these positions as "upward",

161   "horizontal", and "downward" respectively, to represent how each position determines the flower orientation.

162   Within each grid, we aligned the corollas so that their symmetry axes all point in the same direction to minimize

163   the possible effects of variable corolla alignment. For upward and downward presentations, both the longer

164   symmetry axes of disymmetric flowers and the symmetry axes of zygomorphic flowers were aligned parallel to

165   the line connecting the nest and the cage. Moreover, the sharpest vertices of zygomorphic flowers were directed

166   towards the wall opposite to where bees enter the cage. For horizontal presentations, the zygomorphic and

167   disymmetric flowers were aligned so that an approaching bee could view them as upright triangles or upright

168   rectangles, respectively (Fig. 1 d,e).

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170   *Experimental procedures*

171   We used workers from two commercial colonies of bumble bees, *Bombus ignitus* Smith, provided by Agrisect,

172   Ibaraki, Japan. Colonies were maintained in nest boxes. The nest box (one at a time) was connected to a flight

173   cage measuring 100 x 70 x 70 (H) cm through a transparent box equipped with gates, which allowed for the

174   controlled entry and exit of individual bees into and out of the cage (Fig. 1). Pollen was supplied *ad lib* every

175   day, directly into the colony. We used 17 and 18 workers from each colony, respectively.

176   During the training, which was performed before and between the test trials, we allowed the bees to

177   forage freely in the cage by leaving the entrance open. Each training consisted of two phases: initial training

178   phase and advanced training phase. During the initial training phase, a single training grid was placed on the

179   floor of the cage (upward). In the grid, there were four flowers of each symmetry type arranged so that flowers

180   of the same type were not next to each other. The initial phase was used to encourage bees to learn the

181   association between the appearance of the corolla and nectar. Once bees started regular foraging on the training

182   flowers, we proceeded to the advanced training phase, where two training grids were placed in the cage

183   horizontally and downwardly, respectively. We also added six petri dishes (two per corolla shape), which were

184   haphazardly placed on the floor as supplementary feeders. The training flowers and petri dishes were filled with

185   20% (w/w) sucrose solution and were replenished appropriately. Once consistent foraging began, we uniquely

186   marked reliable foragers on their thorax with numbered, coloured tags.

187   Test trials were conducted using a single test grid placed in the cage. For each trial, we randomly

188   selected one of the three symmetry types and arranged 12 of them in the grid, and then selected one of the three

189   orientation types. In other words, one of the nine combination of symmetry and orientation was haphazardly

190   chosen for each trial. As a reward, 10-µL of 30% sucrose solution was used (the concentration was increased to

191   boost the bees' motivation for foraging). A marked bee was selected haphazardly to carry out the trial. Bee

192   foraging was filmed with a video camera (GZ-MG575-S, JVC Kenwood, Yokohama, Japan) that was placed at

193   90° to the flowers. When the bee failed to land on a flower for longer than five minutes or attempted to return to

194   the nest through the gated entrance, we considered the foraging trip to be over; these bees were allowed to return

195   to the nest on its own, or manually taken from the flight cage to the nest.

196   After the experiment, we went through the videotaped images and took a screenshot of each successful

197   landing. A successful landing was defined as the bee's posture being stopped on a flower and her proboscis

198   extended. For each of them, the entry angle was measured using ImageJ (National Institute of Health, Version

199   1.52q, 2019). We measured the angle between two lines extending from the centre of the flower, i.e., one which

200   goes vertically down the middle of the flower and another goes down the midline of the bee's body. We defined

201   the vertical line as a zero degree and measured the counter-clockwise angle of the midline of the bee's body on a

202   0°–360° scales as the directionality of the bee's entry (Fig. 2).

203   We also measured the time it took for the bees to land on each type of flower (hereafter, "landing

204   time") to check if floral symmetry or orientation affects the ease of landing on flowers for bees. This was

205   conducted by placing a video camera at about 90°, 50-80cm away from the flight cage, and recorded the

206   sideview of the grid. We randomly selected three landings per trial and counted the number of frames it took for

207   the bee to land on the flower, using Windows Media Player (30 frames per second). Because a bee usually

208   initiates a hovering phase, approximately 8 mm away from the flower, before moving forwards to land (Reber et

209   al., 2016), we started counting the frames when the bee first hovered in front of the target flower. The bee was

210   considered to be hovering when it stayed in the same position for two or more frames. The counting was

211   continued until the bee touched the flower with either of its legs and the legs remained on the flower during the

212   successive two frames.

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214   *Statistical analysis*

215   We first converted the data of entry angle from degrees to radians. We then calculated the circular variance

216   (mean resultant length, MRL) for each trial, as the ratio of the observed length of the resultant vector to the

217   maximum possible length of resultant vector for the same size of sample. The maximum possible length of

218   resultant vector is obtained when all the entries were in the same direction. For actual computation of MRL, we

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219   used the *rho.circular* function in the R package "circular" (Pewsey et al., 2013). The MRL will take a value

220   from one to zero, which is difficult to interpret in terms of actual angles. Therefore, we converted the MRL into

221   circular standard deviation (circular SD) using the formula:

222      $$\text{circular SD} = \frac{180}{\pi} \cdot \sqrt{-2 \cdot \log(\text{MRL})}, \qquad (1)$$

223   where π is the circular constant. The circular SD, like the usual standard deviation (SD), represents how much

224   any one arbitrary data point deviates from the central value (mean entry angle) in unit of degrees (Pewsey et al.,

225   2013).

226   We then fitted a linear-mixed model (LMM) to the data to determine whether and how floral symmetry

227   and orientation affected the variance of bee's entry angle. The circular SD was used as the response variable.

228   We considered floral symmetry and orientation as fixed effects, bee individual as a random effect, together with

229   an interaction term between symmetry and orientation. A type II Wald Chi-square test was performed to

230   determine the significance of the fixed effects and the interaction.

231   Because we measured the landing time as the number of frames in video image, we fitted a generalised

232   linear-mixed model (GLMM) to this data, using a logarithmic link function and a Poisson error distribution. We

233   calculated the average number of frames elapsed for a landing in each trial, and then used it as a response

234   variable. Floral symmetry and orientations were considered fixed effects, bee individual as a random effect,

235   together with the interaction term between symmetry and orientation. A type II Wald Chi-square test was

236   performed to determine the significance of the fixed effects and interaction.

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238   **Results**

239   We found that the circular SD was significantly affected by orientation (Fig. 3, $\chi^2 = 235.09$, P <0.0001, type-II

240   Wald chi-square test), while it was hardly affected by symmetry or the interaction between symmetry and

241   orientation (symmetry: $\chi^2 = 4.13$, P = 0.17; symmetry x orientation: $\chi^2 = 4.21$, P = 0.38, type-II Wald Chi-square

242   test). The post-hoc test suggests that bees were significantly more consistent in their entry angle on horizontally

243   presented flowers than on upwardly or downwardly presented ones (Fig. 3).

244   On the other hand, the landing time was significantly affected by orientation and the interaction

245   between symmetry and orientation (Fig. 4, orientation: $\chi^2 = 55.94$, P <0.0001, symmetry x orientation: $\chi^2 =$

246 13.11, P = 0.011, type-II Wald Chi-square test), while it was hardly affected by symmetry (χ^2 = 1.51, P = 0.47,

247 type-II Wald Chi-square test). The post-hoc test suggests that bees took significantly longer landing on

248 downwardly presented flowers than on upwardly or horizontally presented ones (Fig. 4).

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250 **Discussion**

251 Bees were observed to enter horizontal flowers at a more consistent angle compared to the other two floral

252 orientations (Fig. 3). In contrast, floral symmetry had little effect on the consistency of bee's entry angle. The

253 interaction between floral symmetry and orientation was not significant, either. We also found no significant

254 effect of floral symmetry on the landing time of bees (Fig. 4). In addition, bees landed almost as quickly on both

255 the horizontal and upwards flowers, while they took significantly longer to land on the downward flowers.

256 Since the publication of Sprengel (1793), it has often been assumed that zygomorphic corollas restrict

257 the movement of a pollinator into the flower (e.g., Armbruster and Muchhala 2020). Based on this assumption,

258 the pollen position hypothesis states that zygomorphy restricts the directionality of approach and movement of

259 pollinators within and between flowers (Leppik 1972; Ostler and Harper 1978; Cronk and Moller 1997).

260 However, our data shows that zygomorphy and disymmetry have little to no effect on the consistency of the

261 bee's entry angle (Fig. 3). Like the radially symmetrical flowers, these flowers allowed the bees to approach

262 from various directions when orientated upwardly or downwardly, resulting in increased variability of their

263 entry angle. Even when presented horizontally, zygomorphy and disymmetry did not increase the stability of the

264 bee's entry compared to actinomorphy (Fig. 3).

265 Our results are inconsistent with those of Culbert and Forrest (2016), who found that bumble bees

266 entered disymmetric flowers at a higher consistency than they did for actinomorphic ones. Although we do not

267 know the reason for this discrepancy, we could at least say that the stabilizing effect of disymmetric flowers

268 found in Culbert and Forrest (2016) was much smaller than that of horizontal flowers in our study: the former

269 found that standard deviations of entry angles were approximately 9° lower in disymmetric than in

270 actinomorphic flowers; in contrast, the latter found that approximately 68° of difference in circular standard

271 deviation of entry angles between horizontal and the other-oriented flowers (mean ± SE of circular SD at

272 horizontal flowers = 22.5 ± 3.8˚, df = 58; upward flowers = 97.2 ± 3.9˚, df = 67; downward flowers = 83.3 ±

273 4.1˚, df = 67, estimated from the fitted GLMM). In other words, the stabilizing effect of floral orientation was

274 more than seven times stronger than that of floral symmetry demonstrated in Culbert and Forrest (2016).

275   The most probable reason why horizontal orientation had the strongest effect on the consistency of the

276   bee's entry angle (Fig. 3) is because gravity forced the bees to fly with the ventral side of their body facing the

277   earth. Fenster et al. (2009) also observed that hovering hummingbirds showed more consistent approaches when

278   flowers were presented horizontally than when the orientation was vertical (upward) or semi-pendant. These

279   observations strongly suggest that the stabilisation effect does not come from visual guidance of the corolla

280   shape or the existence of landing platforms on zygomorphic flowers, but the orientation of flowers relative to

281   the gravity. It has long been known that in nature zygomorphic flowers are typically presented in a horizontal

282   orientation (Ushimaru and Hyodo 2005). Therefore, much of our field impression that pollinators approach to

283   zygomorphic flowers more consistently may have been heavily influenced or mislead by this strong correlation

284   between zygomorphy and horizontality.

285   Our data supports the idea proposed by Fenster et al. (2009) that the stability of pollinator entry angle

286   would be first conferred by the evolution of horizontal flowers that have been driven by abiotic stress, such as

287   rainfall. They also pointed out the possibility that horizontal orientation set the stage for the evolution of

288   symmetry in sexual organs, as a result of its stabilising effect on pollinator entry. It is likely that horizontal

289   presentation of flowers promoted the evolution of symmetric sexual organs in flowers through the increased

290   accuracy of pollen placement. Considering that corolla symmetry had little effect on the stability of entry angle

291   (Fig. 3), however, it seems questionable whether this horizontality eventually lead to the evolution of

292   zygomorphic corolla.

293   Our finding leaves open the question as to why zygomorphic flowers have evolved in the first place,

294   especially in association with horizontal presentation. Given the current information, the most likely

295   evolutionary advantage of zygomorphic corollas would be the restriction of pollinator type, to select for

296   specialised and effective pollinators. This has been supported by empirical evidence (Lázaro and Totland 2014;

297   Yoder et al. 2020). However, it remains unclear if the restriction of pollinator type works better when

298   zygomorphic flowers are presented horizontally.

299   Alternatively, zygomorphic flowers might increase the ease of landing through visual guidance, leading

300   to an increased attractiveness for pollinators. This was unsupported by our landing time data (Fig. 4), in which

301   no significant association was detected between corolla symmetry and landing time. The idea that zygomorphic

302   corolla increases the attractiveness was also unsupported, at least in bumble bees (Culbert and Forrest 2016).

303   There is a possibility that the evolution of zygomorphy may be driven by natural selection acting on the lower

304 lips, serving as a landing platform for pollinators, and the zygomorphic corolla could be a developmental by-

305 product of this selection. Future studies should explore the precedence of horizontality in the evolution of

306 zygomorphy, as well as the selective advantages of a zygomorphic corolla or its associated traits, such as well-

307 developed lower lips. The use of 3D flowers will make it possible to test whether the existence of lower lips on

308 zygomorphic flowers, which are more common in nature than the 2D flowers we used here, could change the

309 results on the effects of the association between zygomorphy and horizontal orientation on the consistency of

310 pollinator's entry angle. Finally, we also urge future studies to quantify the effect of floral symmetry and

311 orientation on pollen transfer to see how plausible the previous assumptions are in terms of actual pollination

312 accuracy.

313   In sum, we first attempted to dissect the entangled effects of corolla symmetry and orientation on the

314 consistency of pollinator's entry angle. We presented compelling evidence that the visual symmetry of corolla

315 has little effect on the consistency of entry angle. Rather, we found that horizontal presentation of flowers plays

316 the largest role in stabilizing pollinator entry. These results may force a reconsideration of the common

317 conception about the evolutionary significance of zygomorphic flowers. That is, zygomorphic corollas have

318 often been assumed to stabilize pollinator's entry to flowers. This could be a misconception caused by the fact

319 that zygomorphic flowers are typically presented at horizontal orientation. That is, horizontal orientation, rather

320 than corolla symmetry, may stabilize entry angle by forcing the pollinator to orient itself relative to gravity. We

321 thus suggest that zygomoprhy in and of itself may not restrict the entry angle of pollinators but may instead

322 allow for the evolution of corolla shape differences which further restrict the entry angle, thus leading to more

323 precise pollen placement. In this respect, our data may support Fenster et al.'s (2009) proposal that horizontal

324 orientation preceded the evolution of zygomorphy. Future studies are needed to determine how the stabilization

325 effect of horizontal presentation affect pollination accuracy, as well as whether and how zygomorphic flowers

326 have evolved and maintained in many angiosperm lineages.

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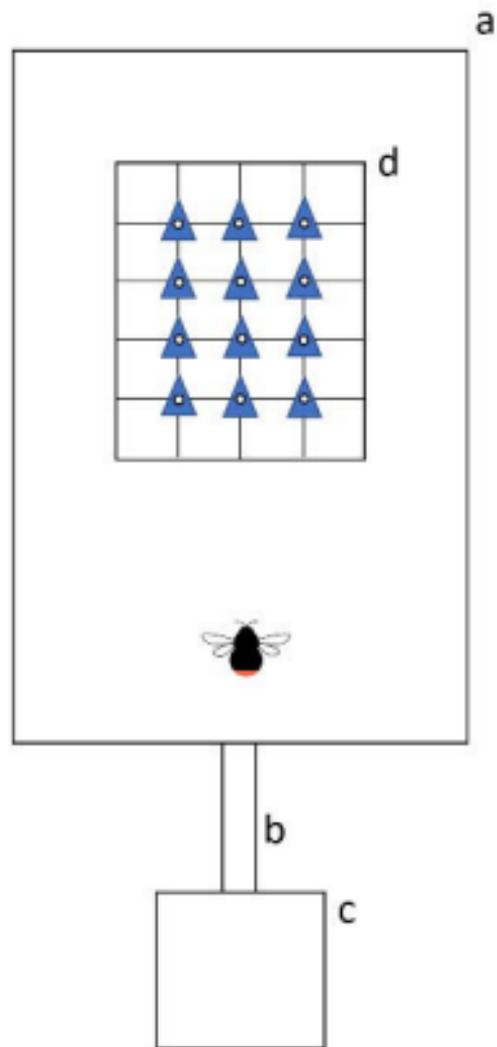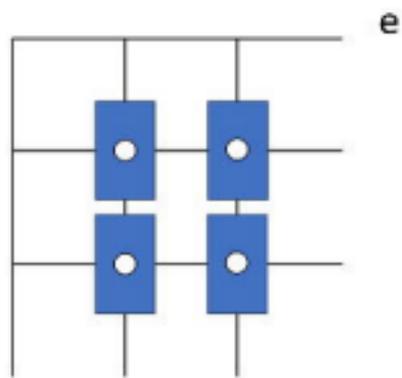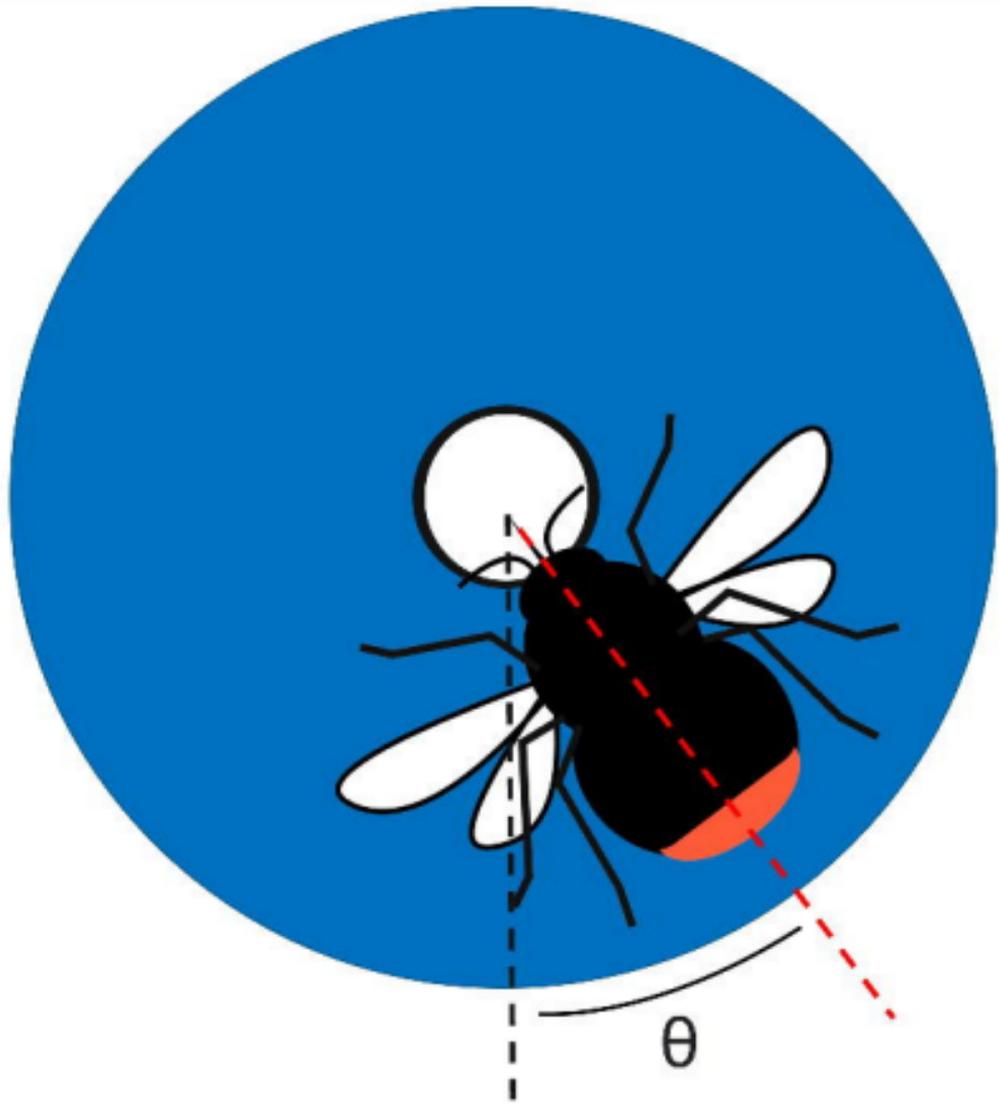398 https://doi.org/10.1186/s12898-016-0080-1

399

400 **Figures**

401 **Fig 1** An aerial view of the experimental set up when flowers were oriented upwardly. Aa: flight cage; b: tunnel

402 for bees to pass through with gated entrance; c: colony; d,e: grid of artificial flowers. The alignments is shown

403 for zygomorphic (d) and dissymmetric (e) flowers, respectively.
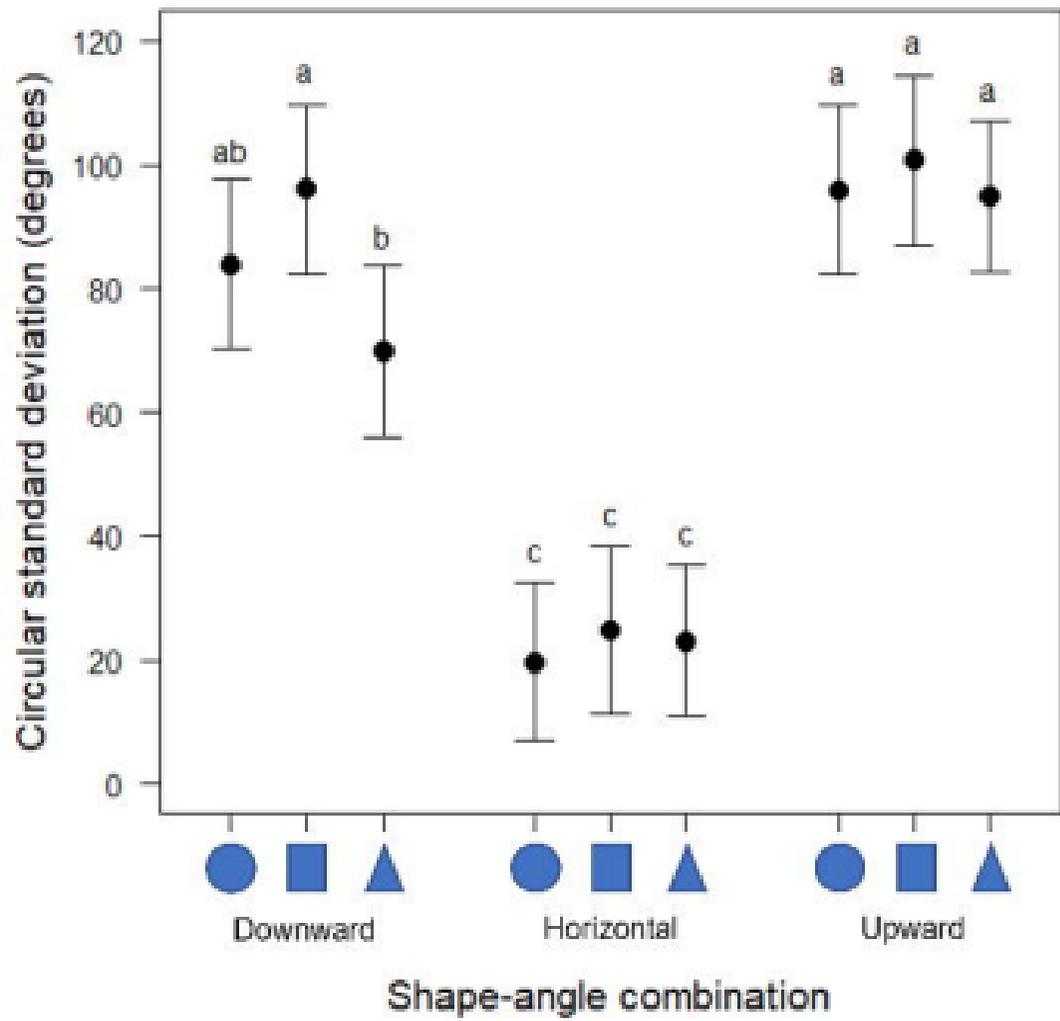
404 **Fig 2** Schematic representation of the method used for measuring the entry angle of a bee. Two dotted lines

405 were drawn from the centre of the artificial flower, one going down the middles of the flower (black) and

406 another down the midline of the bee. We defined the black line as a zero degree and measured the counter-

407 clockwise angle between these lines on a 0-360°scale as the bee's entry angle.

408 **Fig 3** The circular SD of pollinator entry angle in nine shape-angle combinations. The shapes present on the X

409 axis represent radial symmetry, bilateral symmetry, and zygomorphy, respectively. The data is divided into three

410 groups; (a), (b), (c). Error bars indicate the standard error of the circular SD for each combination. Means with

411 shared letters indicate that there is no significant difference at a 0.05 alpha level. Significance levels are adjusted

412 by Tukey correction. The data indicates that horizontal orientation results in a lower circular SD, indication less

413 variance in entry angle compared to other orientations. Upward and downward facing artificial flowers have a

414 similar circular SD, however radial-downward and bilateral-downward showed slightly lower circular SD

415 compared to other combinations of upward or downward flowers.

416 **Fig 4** The average landing time (in number of frames) in nine shape-angle combinations. The shapes present on

417 the X axis represent radial symmetry, bilateral symmetry, and zygomorphy, respectively. The data is divided

418 into four groups; (a), (b), (c), (d). Error bars indicate the standard error of the circular SD for each combination.

419 Means with shared letters indicate that there is no significant difference at a 0.05 alpha level. Significance levels

420 are adjusted by Tukey correction. The data indicates that horizontal and upward facing flowers have lower mean

421 landing times, while downward facing flowers had the longer landing times.