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1   Flower orientation enhances pollen transfer in bilaterally symmetrical flowers

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17

18   Abstract

19   Zygomorphic flowers are usually more complex than actinomorphic flowers and are

20   more likely to be visited by specialized pollinators. Complex zygomorphic flowers tend

21   to be oriented horizontally. It is hypothesized that a horizontal flower orientation ensures

22   effective pollen transfer by facilitating pollinator recognition (the recognition-facilitation

23   hypothesis) and/or pollinator landing (the landing-control hypothesis). To examine these

24   two hypotheses, we altered the angle of *Commelina communis* flowers and examined the

25   efficiency of pollen transfer, as well as the behavior of their visitors. We exposed

26   unmanipulated (horizontal-), upward-, and downward-facing flowers to syrphid flies

27   (mostly *Episyrphus balteatus*), which are natural visitors to *C. communis*. The frequency

28   of pollinator approaches and landings, as well as the amount of pollen deposited by *E.*

29   *balteatus* decreased for the downward-facing flowers, supporting both hypotheses. The

30   upward-facing flowers received the same numbers of approaches and landings as the

31   unmanipulated flowers, but experienced more illegitimate landings. In addition, the

32   visitors failed to touch the stigmas or anthers on the upward-facing flowers, leading to

33   reduced pollen export and receipt and supporting the landing-control hypothesis.

34 Collectively, our data suggested that the horizontal orientation of zygomorphic flowers

35 enhances pollen transfer by both facilitating pollinator recognition and controlling

36 pollinator landing position. These findings suggest that zygomorphic flowers which

37 deviate from a horizontal orientation may have lower fitness because of decreased pollen

38 transfer.

39 **Keywords** *Commelina communis*, pollen transfer, pollinator specialization, horizontal

40 flower orientation, zygomorphic flower

41

42 **Introduction**

43 Pollinator specialization is presumed to be an essential selective force for floral diversity

44 in angiosperms (Darwin 1862; Grant and Grant 1965; Stebbins 1970; Fenster et al. 2004;

45 Kay et al. 2006). Many reproductive characteristics, such as floral colors and scents, long

46 corolla tubes, nectar spurs, and flowering phenology, are adaptations to specific

47 pollinator groups (Nilsson 1998; Johnson and Steiner 2000). In particular, bilaterally

48 symmetrical (zygomorphic) flowers have been repeatedly derived from radially

49 symmetrical (actinomorphic) flowers (Neal et al. 1998 and references therein; Wolfe and

50 Krstolic 1999; Sargent 2004). Fenster et al. (2004) reanalyzed Robertson's (1928) data

51 and found that, compared to actinomorphic flowers, zygomorphic flowers were more

52 frequently visited by only one functional group of pollinators, suggesting that a

53 specialized pollination system is prevalent in zygomorphic flowers.

54   Neal et al. (1998) reported that the *en face* surface of zygomorphic flowers is

55 usually vertical or oblique (i.e., the orientation of the main floral axis is nearly horizontal,

56 Fig. 1) and two hypotheses have been proposed to explain this horizontal flower

57 orientation (Neal et al. 1998; Ushimaru and Hyodo 2005). First, zygomorphic flowers are

58 usually more complex than actinomorphic flowers. Because of this morphological

59 complexity, zygomorphic flowers should facilitate recognition and memorization of

60 complex floral patterns by specialized pollinators (Neal et al. 1998; West and Laverty

61 1998). Facilitating recognition, in turn, allows the plant to receive repeated pollinator

62 visits. Neal et al. (1998) argued that the horizontal orientation of the floral axis in

63 zygomorphic flowers could restrict the approach course of visiting insects to facilitate

64 their recognition of complex floral patterns (the recognition-facilitation hypothesis;

65   originally introduced as the unequal image projection hypothesis in Neal et al. 1998).

66   Second, the morphological complexity of zygomorphic flowers should restrict pollinators

67   to landing sites where they contact the anthers and stigmas correctly, ensuring pollen

68   transfer (Ushimaru et al. 2007). The horizontal flower orientation can control access to

69   landing sites on zygomorphic flowers (the landing-control hypothesis; Ushimaru and

70   Hyodo 2005; see also the natural position hypothesis and the pollen position hypothesis

71   in Neal et al. 1998). Under both of these hypotheses, zygomorphic flowers facilitate their

72   own pollination by orientating their main axis horizontally.

73   Despite the hypothesized importance of a horizontal orientation for pollen

74   transfer in zygomorphic flowers, its significance for successful pollen export and receipt

75   has not been tested in the field. The effects of flower angle on pollinator behaviors, pollen

76   export and receipt, and fruit set have been examined mainly in upright-oriented or

77   pendant actinomorphic flowers (Fulton and Hodges 1999; Tadey and Aizen 2001). Fulton

78   and Hodges (1999) showed that artificial changes in floral angle diminished pollinator

79   visitation in *Aquilegia pubescens*, but they did not examine pollen transfer. Tadey and

80   Aizen (2001) revealed that changes in flower (inflorescence) angle reduced pollen receipt

81   on the stigmas in *Tristerix corymosus*, but the number of pollinator visits and pollen

82   export were unaffected. In zygomorphic flowers, our previous study revealed a

83   significant effect of flower angle on pollinator landings in *Commelina communis*,

84   supporting the landing-control hypothesis (Ushimaru and Hyodo 2005). However, we did

85   not examine pollen transfer. Thus, our understanding of the adaptive significance of

86   flower orientation, especially in terms of pollen transfer in zygomorphic flowers, is still

87   incomplete; further examination is needed to elucidate how the artificial manipulation of

88   flower orientation alters pollinator behaviors (approach and landing frequency and

89   landing site) and how pollen transfer is affected by these behavioral changes.

90   Here, we report the results of a field experiment in which we examined two

91   major hypotheses concerning the function of horizontal orientation on pollen transfer in

92   zygomorphic flowers: the recognition-falicitation hypothesis and the landing-control

93   hypothesis. Note that these two hypotheses are not mutually exclusive but do lead to

94   different predictions for several aspects of the pollination process, which allows us to

95   determine the relative importance of the two hypothesized processes in the pollination

96   success of zygomorphic flowers. The recognition-facilitation hypothesis predicts that

97   changes in the floral angle upward or downward should reduce the number of approaches

98   by visitors. In comparison, the landing-control hypothesis predicts that changes in the

99   floral angle will impair the function of landing-specialized organs, which may increase

100   visitor landings on the floral organs unsuitable for pollination (i.e., illegitimate landings)

101   (Ushimaru and Hyodo 2005). The recognition-facilitation hypothesis and the

102   landing-control hypothesis both predict that pollen transfer decreases in experimental

103   flowers through decreased visitation frequency and through decreased frequency of

104   legitimate landings, respectively. We experimentally altered the orientation of *C.*

105   *communis* flowers to examine how the floral angle affects the approach and landing

106   behavior of, and pollen transfer by, insect pollinators. Based on our results, we discuss the

107   validity of the recognition-facilitation and landing-control hypotheses in horizontally

108   oriented zygomorphic flowers.

109

110   **Materials and methods**

111

112   Study species

113

114   *Commelina communis* L. (Commelinaceae) is an annual, andromonoecious herb found

115   throughout temperate northeast Asia, often growing around or near rice fields and roads.

116   A single plant usually has several inflorescences in which perfect flowers bloom before

117   staminate flowers. Each inflorescence contains three or four flower buds inside the bract.

118   In most cases, one flower per inflorescence opens each day at sunrise and lasts until noon

119   of the same day.

120   The flowers of *C. communis* have three types of stamens: two long brown

121   stamens, one medium-length yellow stamen, and three short yellow stamens. Anthers of

122   the long (L-anther) and medium-length (M-anther) stamens produce fertile pollen,

123   whereas anthers of the short stamens (S-anther) produce only a small amount of sterile

124   pollen (Morita and Nigorikawa 1999). Pollen production on the M- and L-anthers does

125   not differ between perfect and staminate flowers and among different-positioned flowers

126   (Morita and Nigorikawa 1999). The flowers of *C. communis* do not bear nectar; thus,

127   pollen is the only floral reward for their pollinators. Pollen from the L-anthers is believed

128   to contribute to outcrossing, whereas pollen from the M-anthers functions mainly as a

129 reward for pollinators (Vogel 1978; Faden 1992). In *C. communis*, filaments of the long

130 stamens are typically elongate, and they seem to function as a landing platform (Fig. 1;

131 Ushimaru and Hyodo 2005; Ushimaru et al. 2007). Ushimaru et al. (2003a, b) suggested

132 that the position of the stigmas evolved to maximize the receipt of pollen grains from the

133 L-anthers, which are situated at the same height in perfect flowers (Fig. 1).

134   *Commelina communis* is self-compatible and exhibits both delayed autogamy

135 and infrequent bud pollination within a single flower (Morita and Nigorikawa 1999;

136 Ushimaru et al. 2007). The pistils and long stamens roll up at the end of anthesis,

137 facilitating contact between the L-anther and stigma and subsequent autonomous

138 self-pollination (Morita and Nigorikawa 1999). However, the fact that the pollen:ovule

139 ratio in the perfect flowers is within the range of those in other facultatively xenogamous

140 species (Cruden 1977; Morita and Nigorikawa 1999) and staminate flowers are present in

141 addition to perfect flowers suggests that pollinator-mediated outcrossing occurs in *C.*

142 *communis*. Syrphid flies (*Episyrphus balteatus*) are the exclusive visitors to the flowers of

143 *C. communis* in many fields, although bee species, such as bumblebees, honeybees, and

144 small solitary bees, sometimes visit as well (Ushimaru and Hyodo 2005; Ushimaru et al.

145 2007; Uchihashi H and Ushimaru A unpublished data).

146

147 Field experiment

148

149 We conducted a field experiment using a population of *C. communis* in Iwakura, Kyoto,

150 Japan (35°05'N, 135°47'E), in September 2004. Native syrphid flies (*E. balteatus*) were

151 frequent visitors to the flowers of *C. communis* plants, and some smaller syrphid species

152 visited the flowers infrequently. We experimentally prepared three types of flowers that

153 differed in terms of their floral angle (Fig. 1):

154 (1) 'Unmanipulated': intact flowers with angles of 0 to 20°;

155 (2) 'Up': flowers whose faces were turned upward; and

156 (3) 'Down': flowers whose faces were turned downward.

157

158 We used only perfect flowers in our experiment to avoid the effect of sexual differences in

159 petal size on pollinator behavior (Ushimaru and Hyodo 2005). For each trial, we

160 arbitrarily chose three neighboring perfect flowers from a single flower patch, which

161 consisted of one to three individuals. We then manipulated the orientation of the flowers

162 (Unmanipulated, Up, or Down) before pollinators had access to them and observed the

163 approach and landing behavior of the pollinators for 15 min. It was technically difficult to

164 change the stalk angle of an individual flower inside a bract; therefore, we leaned the

165 plant stems and fixed them to create Up flowers, and we bent the inflorescence stalks with

166 staples to create Down flowers (Fig. 1); the angles of these flower types were

167 approximately 90° and –90°, respectively. We often removed a single leaf to maintain a

168 space in front of Down flowers. In our previous study, the leaf removal treatment did not

169 induce discrimination among experimental flowers by syrphid flies (Ushimaru & Hyodo

170 2005) and seemed not to affect their landing behavior. All trials were conducted in the

171 morning (06:00–09:30 h), and a new flower patch was used for each trial. In total, 57

172 trials were conducted during the experimental period, which allowed us to examine 57

173 Unmanipulated, 57 Up, and 57 Down flowers.

174

175 Pollinator behavior

176

177 We first analyzed two types of pollinator behavior: approaching and landing.

178 Approaching occurred when pollinators found flowers and approached them from the

179 front. Landing was defined as a pollinator landing on any part of a flower after

180 approaching it. We then split pollinator landing events into the following three types

181 (Ushimaru and Hyodo 2005):

182 (1) Legitimate landing: the pollinator held on to the filaments of the L- and M-anthers or

183   a small white petal beside the anthers and foraged or collected pollen grains from the

184   M-anther, while simultaneously touching the L-anthers or stigma with the ventral

185   surface of its abdomen (Fig. 1);

186 (2) L-anther landing: a syrphid fly hung onto the L-anthers and ingested pollen from them

187   directly (Fig. 1); and

188 (3) Petal and side landing: the pollinator foraged for pollen from the M- or S-anthers after

189   landing on the blue petals or on the bracts or sepals at the side of the flower. Because

190   the L-anthers and sitgma are at the bottom of a flower, syrphid flies on the blue petals

191   and bracts rarely touch these organs in Up flowers (Fig. 1, Ushimaru et al. 2007). For

192   Down flowers, they mostly land on the back side of the blue petals and never touch

193   the L-anthers and the stigma.

194

195   In our analyses, we considered data from 57 observations. We counted the number of

196   approaches and landings by pollinators on each flower type during each observation

197   period. During the experiment, we recorded 254, 34, 4, 2, and 2 approaches by *E.*

198   *balteatus*, smaller syrphid flies, *Bombus diversus*, a small-sized bee, and a hawkmoth,

199   respectively; however, we analyzed only the data for *E. balteatus* and other small syrphid

200   flies. Most *E. balteatus* individuals consistently visited *Commelina* flowers and

201   infrequently visited flowers of other species during the morning at the study site

202   (Ushimaru A., personal observation).

203   *Episyrphus balteatus* individuals were sometimes observed landing on a flower

204   repeatedly (e.g., petal landing -> legitimate landing -> L-anther landing) during a single

205   visitation, but we counted this as one landing. When both legitimate and other landings

206   were observed in a single bout, we used the first landing for analyses. For example, when

207   a syrphid fly was observed to land on the legitimate position and then on the L-anther in a

208   single bout, we defined the landing type as legitimate landing. The landing repetitions

209   were seen 15 times out of a total of 124 landings of *E. balteatus* and only once out of 23

210   landings of the smaller syrphid flies.

211   To test the recognition-facilitation and landing-control hypotheses, we

212   analyzed how changes in flower orientation changed the behavior of visitors. We first

213   compared the number of approaches and the total number of landings between

214   Unmanipulated and manipulated (Up or Down) flowers using generalized linear models

215   (GLMs) with Poisson error (logarithmic link). For these analyses, the number of

216   behaviors (approaches and landings) were treated as the response variable and the

217   treatment type as a fixed effect. We analyzed the behavior of *E. balteatus* and the smaller

218   syrphid fly species separately. A significant decrease in the numbers of approaches and

219   landings in the Up and Down flowers relative to the Unmanipulated flowers would

220   support the recognition-facilitation hypothesis.

221   To test the effect of flower orientation on whether landing occurs after approach

222   or not, a GLMM analysis with binomial errors and logit-link function was applied. The

223   model has two explanatory variables, treatment (Unmanipulated, Up, and Down) as a

224   fixed effect, and flower identity as a random term, because flower identity was the source

225   of replication. In the analyses, we only used data from flowers that received one or more

226   approaches. The behavior of *E. balteatus* and the smaller syrphid fly species were

227   separately analyzed. The recognition-facilitation hypothesis predicts no difference in the

228   occurence of landing after approach among experimental flowers, whereas the

229   landing-control hypothesis predicts significant decreases in the occurence of landing after

230   approach in the Up and Down flowers relative to the Unmanipulated flowers (Ushimaru

231   and Hyodo 2005).

232   We then used GLMMs with binomial errors and the logit-link function to

233   examine the effect of treatment (Up or Down) on legitimate landing. In the models, the

234   presence of legitimate landing (presence, 1; non-legitimate landing, 0) was considered a

235   response variable. The explanatory variables were treatment (fixed effect) and flower

236   identity (random term). We only used data of flowers that received one or more landings

237   in the analyses. We also compared the ratios of three landing types (legitimate, L-anther,

238   and petal and side landings) to the total observed landings between Unmanipulated and

239   treated (Up or Down) flowers using chi-squared and Fisher's exact tests. We did not apply

240   these analyses to data from the smaller syrphids because of the small sample size.

241   Significant decreases in the number of legitimate landings both in the Up and Down

242   flowers would provide support for the landing-control hypothesis.

243

244   Pollen transfer

245

246   We examined the effect of flower orientation on pollen removal and deposition. We

247   collected the M- and L-anthers and stigmas from the Unmanipulated, Up, and Down

248   flowers (n = 26 for each), which were exposed to pollinators from bud break until 10:00 h.

249   The sampled flowers were randomly selected from the flowers for which pollinator

250   behaviors were observed. Additionally, we collected the M- and L-anthers and stigmas

251   from ten non-visited flowers (i.e., newly opened flowers) at 06:00 h in order to compare

252   pollen transfer with the Unmanipulated flowers. We stored each sample separately in 0.1

253   mL 70% ethanol. Because the pollen grains detach easily from the anthers in solution, we

254   estimated the number of pollen grains per anther by counting the number of grains in

255 three 5.0-μL aliquots per sample under a light microscope (×85). We directly counted the

256 number of pollen grains on the stigmas in each sample using the microscope. *Commelina*

257 species have both large and small pollen grains, but because the small grains are less

258 abundant and generally not viable (Hrycan and Davis 2005; Ushimaru et al. 2007), we

259 counted only the number of large grains. Male reproductive success has often been

260 estimated by counting the number of pollen grains remaining on the anthers (e.g. Ishii and

261 Sakai 2002; Ishii 2004).

262   We then compared the number of pollen grains on the M- and L-anthers and

263 stigmas of the Non-visited, Up, or Down flowers to those of the Unmanipulated flowers

264 using generalized linear models (GLMs) with Poisson error (logarithmic link). Statistical

265 analyses were done using the statistical software package R (R Development Core Team

266 2005).

267

268 **Results**

269

270 Pollinator behavior

271

272 Compared to the Unmanipulated flowers, approaches by *E. balteatus* to the experimental

273 flowers decreased only in the Down treatment (GLM, Up: $b = -0.253 \pm 0.149$, $z = -1.70$, P

274 $= 0.089$; Down: $b = -0.372 \pm 0.154$, $z = -2.41$, $P = 0.016$; a negative value for b implies

275 that the treatment has a negative effect on the number of behaviors; Fig. 2). A significant

276 decrease in the frequency of landings and in the occurence of landing after approach was

277 also found only in the Down flowers (landing: GLM, Up: $b = -0.097 \pm 0.197$, $z = -0.49$, P

278 $= 0.62$; Down: $b = -0.944 \pm 0.257$, $z = -3.67$, $P < 0.001$; landing after approach: GLMM,

279 Up: $b = 0.313 \pm 0.361$, $z = 0.87$, $P = 0.39$; Down: $b = -1.134 \pm 0.415$, $z = -2.74$, $P = 0.006$).

280   Legitimate landings decreased significantly in the Up and Down flowers

281 relative to the Unmanipulated flowers (GLMM, Up: $b = -3.077 \pm 0.857$, $z = -3.59$, $P <$

282 0.001; Down: $b = -1.746 \pm 0.778$, $z = -2.24$, $P = 0.025$). For the Up flowers, L-anther

283 landings, and petal and side landings were observed less and more frequently than for the

284 Unmanipulated flowers, respectively ($\chi^2 = 70.78$, $df = 2$, $P < 0.001$; Fig 3). The ratio of

285 L-anther landings to total landings was unchanged but that of petal and side landings

286 increased in the Down flowers (Fisher's exact test, $P < 0.001$; Fig. 3).

287 We found no significant difference in the number of approaches and landings,

288 and the proportion of approaching to landing by smaller syrphid flies between

289 experimetal flowers (GLMM, $P > 0.05$ for all fixed effects). We observed only 23

290 landings by smaller syrphid flies (five, six, and 12 landings on the Unmanipulated, Up,

291 and Down flowers, respectively), and most of them were petal and side landings.

292

293 Pollen transfer

294

295 Seventy-four percent of pollen grains were removed from the M-anthers of

296 Unmanipulated flowers during the experimental period (Unmanipulated vs. Non-visited,

297 GLM, $b = 1.362 \pm 0.029$, $z = 46.23$, $P < 0.001$; Fig. 4A). Significantly fewer pollen grains

298 were removed from the M-anthers of Up and Down flowers, relative to Unmanipulated

299 flowers (Up: $b = 0.838 \pm 0.027$, $z = 30.66$, $P < 0.001$; Down: $b = 1.015 \pm 0.027$, $z = 38.103$,

300 $P < 0.001$). This indicates that manipulation of floral orientation decreased pollen

301 removal from the M-anther (41% and 29% removed from the Up and Down flowers,

302 respectively; Fig. 4A).

303 Seventy-one percent of pollen grains were removed from the L-anthers in the

304 Unmanipulated flowers during the experimental period (Unmanipulated vs. Non-visited,

305 GLM, $b = 1.221 \pm 0.016$, $z = 77.28$, $P < 0.001$; Fig. 4B). Significantly fewer pollen grains

306 were removed from the L-anthers of Up flowers, compared to Unmanipulated flowers

307 (30% removed; $b = 0.858 \pm 0.014$, $z = 60.49$, $P < 0.001$), whereas there was no difference

308 in the amount of pollen removed from the L-anthers between the Down and

309 Unmanipulated flowers (70% removed in the Down flowers: $b = 0.029 \pm 0.017$, $z = 1.71$,

310 $P = 0.087$; Fig. 4B).

311 The stigmas of the Unmanipulated flowers received about 20 pollen grains

312 during the experimental period (Fig. 4C), and this increase from the Non-visited flowers

313 was significant (GLM, $b = -2.030 \pm 0.190$, $z = -10.67$, $P < 0.001$). The stigmas of the Up

314 and Down flowers received significantly fewer pollen grains than those of the

315 Unmanipulated flowers (Up: $b = -0.420 \pm 0.066$, $z = -6.34$, $P < 0.001$; Down: $b = -0.402 \pm$

316   0.066, z = -6.10, P < 0.001).

317

318   **Discussion**

319

320   We examined two hypotheses regarding the adaptive significance of the horizontal

321   orientation in zygomorphic flowers: the recognition-facilitation hypothesis and the

322   landing-control hypothesis. These hypotheses predict different patterns of pollen transfer

323   success: the former predicts that pollen transfer will be correlated with total visitation

324   frequency, whereas the latter predicts association with the frequency of legitimate

325   landings. The results of our experiments using zygomorphic *C. communis* flowers that

326   were mainly visited by *E. balteatus* support both hypotheses, although there was more

327   support for the landing-control hypothesis.

328   We found support for the recognition-facilitation hypothesis, but only from the

329   comparison between horizontally oriented and downward-facing flowers. The total

330   frequencies of pollinator approach and landing, and the proportion of approaches to

331   landings decreased only in the Down flowers relative to the Unmanipulated flowers (Fig.

332   2), while the recognition-facilitation hypothesis predicted a decrease in both the Up and

333   Down treatments. Moreover, the recognition-facilitation hypothesis predicts that the

334   number of pollinator visits should be reflected in pollen transfer success: i.e., pollen

335   export and receipt would decrease only in the Down flowers. However, our results

336   showed that pollen removal from the M- and L-anthers and pollen deposition on the

337   stigma consistently decreased in the Up flowers (Fig. 4). Although the Down flowers also

338   experienced decreased pollen removal from the M-anther and pollen deposition on the

339   stigma, there was no difference in pollen removal from the L-anther between the Down

340   and Unmanipulated flowers (Fig. 4b). Thus, the recognition-facilitation hypothesis is

341   only weakly supported for the stages of pollen export and receipt.

342   Results of the landing type analyses strongly supported the landing-control

343   hypothesis. The manipulation of flower angle significantly changed the composition of

344   landing types and decreased the frequency of legitimate landings in the Up and Down

345   flowers (Fig. 3). The landing-control hypothesis predicts that the frequency of legitimate

346   landings should be reflected in the pollen transfer success, that is, pollen export and

347   receipt should also decrease in the Up and Down flowers. As expected, pollen removal

348   from the M- and L-anthers and pollen deposition on the stigma decreased in both the Up

349   and Down flowers compared to the Unmanipulated flowers. Pollen removal from the

350   L-anthers in the Down flowers, however, showed no difference to the Unmanipulated

351   flowers (Fig. 4). No difference in pollen removal from the L-anthers between the Down

352   and Unmanipulated flowers can be explained by our finding that the Down flowers

353   experienced relatively frequent L-anther landings (Fig. 3) and pollen predation from the

354   L-anthers. As a result, the amount of pollen removal was almost equal to the

355   Unmanipulated flowers. Since our experiments cannot discriminate between pollen

356   removal for pollen predation and for plant reproduction, our data on pollen removal may

357   include these two effects. This suggests that the amount of pollen effectively transferred

358   for plant reproduction could be overestimated, especially in the Down flowers.

359   Meanwhile, the stigmas of the Up and Down flowers received significantly fewer pollen

360   grains than the Unmanipulated flowers. This result indicates that illegitimate (L-anther,

361   petal, and side) landings contribute less to pollen deposition. Collectively, our results

362   suggest that manipulation of flower orientation results in a decrease in legitimate landings

363   relative to illegitimate landings, and, consequently, reduced pollen export and receipt,

364   providing more support for the landing-control hypothesis at the stage of pollen transfer.

365   Traits that increase pollen export and receipt are generally favored by natural

366   selection (Harder and Wilson 1994; 1998). Pollen transfer efficiency depends on the

367   physical fit between pollinators and the mating-related floral organs (i.e., anthers and

368   stigmas), which are usually in specific positions in zygomorphic flowers (e.g., at the top

369   or bottom of the flower or inside the guard petals or corolla tubes; Nilsson 1988; Harder

370   and Barrett 1993; Johnson and Steiner 1995; Wilson 1995; Ushimaru and Nakata 2001).

371   Our results demonstrate that landing on a legitimate position is important for successful

372   pollination in *C. communis*, presenting the evidence for the functional importance of the

373   structure and orientation of zygomorphic flowers in terms of controlling the landing sites

374   of visitors on the flower.

375   The adaptive significance of flower orientation has been investigated in terms

376   of pollinator attraction (Kevan 1975; Kudo 1995; Huang et al. 2002; Patino et al. 2002;

377   Ushimaru et al. 2006), but few studies have focused on the efficiency of pollen transfer

378   (e.g., Tadey and Aizen 2001; Ushimaru et al. 2006). We found that flower angle strongly

379   influenced pollinator behaviors and consequent pollen transfer in a zygomorphic species,

380   suggesting the importance of flower orientation in specialized pollination systems. In

381 plants with actinomorphic flowers, flower angle affects pollinator behavior and/or

382 pollination efficiency in a specialized (hawkmoth and humming bird) pollination system

383 (Fulton and Hodges 1999; Tadey and Aizen 2001), but not in a generalized pollination

384 system (Huang et al. 2002). Thus, further research is warranted to test the generality of

385 the landing-control hypothesis, which should focus on the function of flower orientation

386 in controlling pollinator landing behavior and pollen transfer efficiency, with respect to

387 both floral symmetry (zygomorphic or actinomorphic) and pollination systems

388 (specialized or generalized). We predict that in zygomorphic plants with a generalized

389 pollination system, flower orientation would less strictly regulate pollinator landing and

390 pollen transfer because of the diverse reponses of different pollinators (which may differ

391 in body size and foraging behavior) to the flower orientation.

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479   Figure legends

Fig. 1
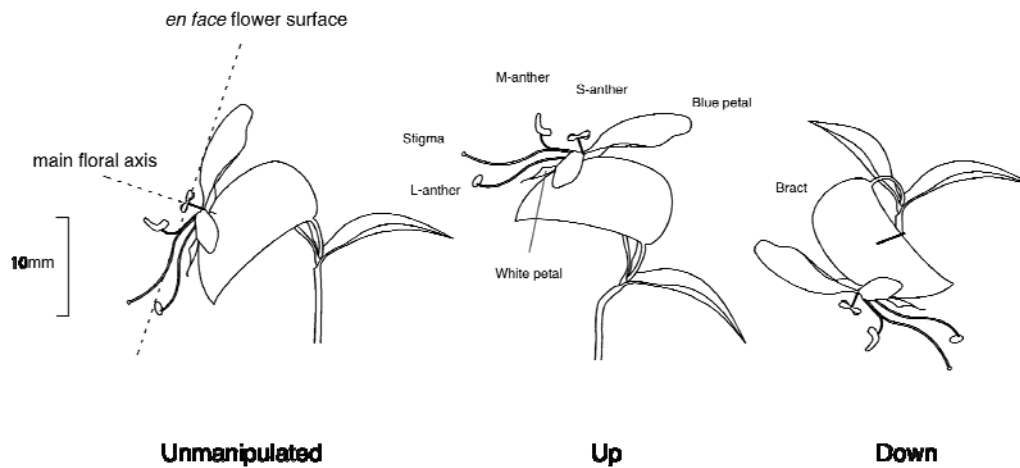
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481   Fig. 1. Side views of experimental flowers of *Commelina communis*: intact

482   (Unmanipulated), upward-facing (Up), and downward-facing (Down) flowers. Main

483   floral axis of Unmanipulated and Manipulated (Up and Down) flowers is nearly

484   horizontal and vertical, respectively. Positions of the stigma, S-, M- and L-anthers, and

485   blue and white petals within a flower are illustrated. The plant stem was bent and fixed to

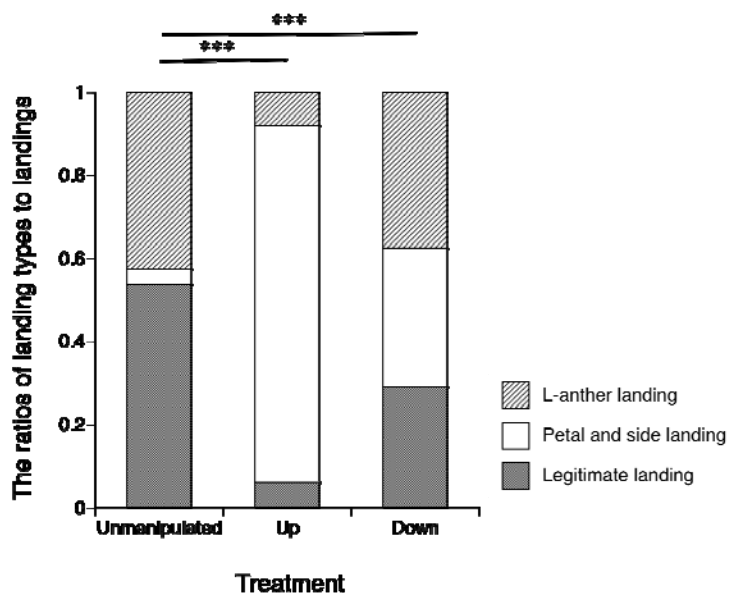486   prepare Up flowers. Bracts of Down flowers were fixed with staples.

Fig. 2

(a) Approach

(b) Landing

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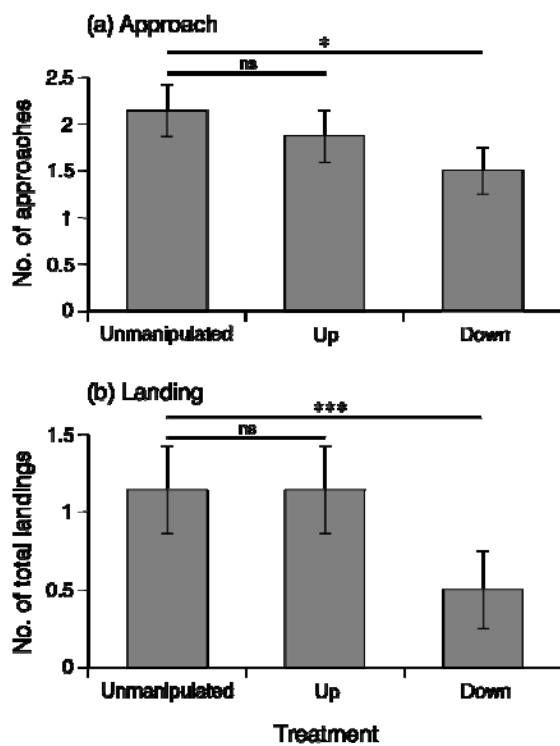
Fig. 2. Mean number of a) approaches to and b) total landings per flower (per 15 min, n =
57) by *Episyrphus balteatus* on the experimental (Unmanipulated, Up, and Down)
flowers of *Commelina communis*. Bars show standard errors. * $P < 0.05$,
*** $P < 0.001$, ns P > 0.05 by GLM or GLMM (see text).

Fig. 3. The ratio of legitimate, L-anther, and petal and side landings to total landings by *Episyrphus balteatus* for the experimental (Unmanipulated, Up, and Down) flowers. The number of total landings for the Unmanipulated, Up, and Down flowers were 54, 49, and 21, respectively. *** $P < 0.001$ by chi-squared test (Unmanipulated vs. Up) and Fisher's exact test (Unmanipulated vs. Down).

Fig. 4

(a) M-anther

(b) L-anther

No. of pollen grains

(c) Stigma

Treatment
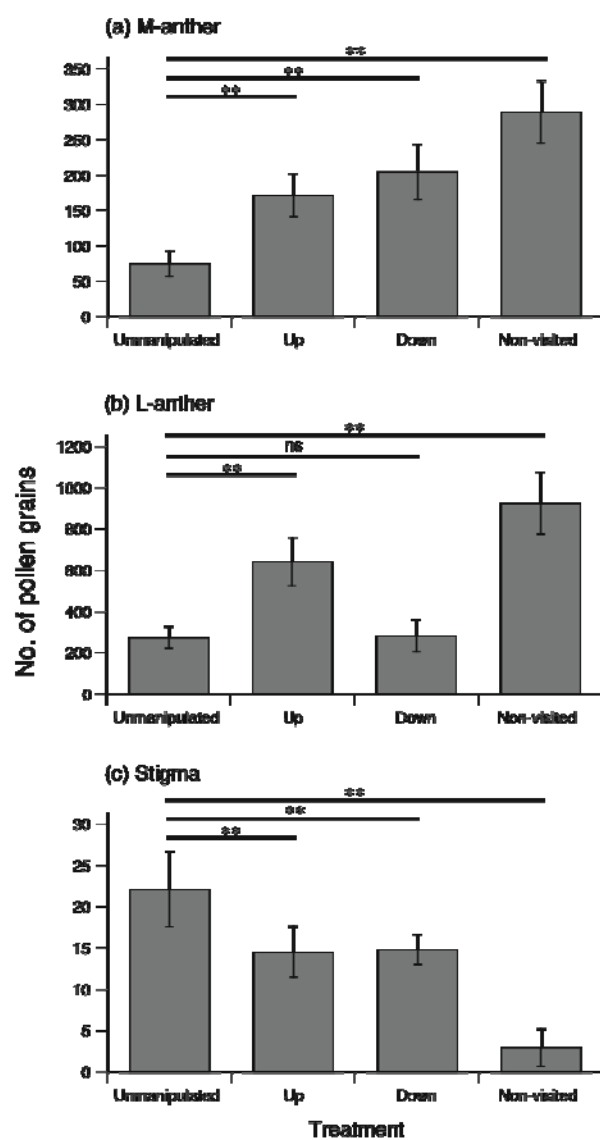
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500   Fig. 4. Mean number of remaining pollen grains on the a) M-anther and b) L-anther, and

501   pollen deposited on the c) stigma for the experimental (Unmanipulated, Up, and Down)

502   and non-visited flowers. Bars show standard errors. ** $P < 0.01$, ns $P > 0.05$ by GLMs

503   (see text).

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