Gynomonoecy in a mycoheterotrophic orchid *Eulophia zollingeri* with autonomous selfing hermaphroditic flowers and putatively outcrossing female flowers

Kenji Suetsugu[1]

[1] Department of Biology, Graduate School of Science, Kobe University, Kobe, Japan

Corresponding Author:

Kenji Suetsugu[1]

Department of Biology, Graduate School of Science, Kobe University, 1-1 Rokkodai, Nada-ku, Kobe, 657-8501, Japan

Email address: kenji.suetsugu@gmail.com

Abstract

Most orchid species exhibit an extreme case of hermaphroditism, owing to the fusion of male and female organs into a gynostemium. Exceptions to this rule have only been reported from the subtribes Catasetinae and Satyriinae. Here, I report an additional orchidaceous example whose flowers are not always hermaphroditic. In Japanese populations of *Eulophia zollingeri* (Rchb.f.) J.J.Sm, a widespread Asian and Oceanian orchid, some flowers possess both the anther (i.e., anther cap and pollinaria) and stigma, whereas others possess only the stigma. Therefore, pollination experiments, an investigation of floral morphology and observations of floral visitors were conducted to understand the reproductive biology of *E. zollingeri* in Miyazaki Prefecture, Japan. It was confirmed that *E. zollingeri* studied here possesses a gynomonoecious reproductive system, a sexual system in which a single plant has both female flowers and hermaphroditic flowers. In addition, hermaphroditic flowers often possessed an effective self-pollination system while female flowers could avoid autogamy but suffered from severe pollinator limitation, due to a lack of agamospermy and low insect-mediated pollination. The present study represents the

30   first documented example of gynomonoecy within Orchidaceae. Gynomonoecy in *E. zollingeri*

31   may be maintained by the tradeoff in reproductive traits between female flowers (with low fruit

32   set but potential outcrossing benefits) and hermaphroditic flowers (with high fruit set but

33   inbreeding depression in selfed offspring). This mixed mating is probably important in

34   mycoheterotrophic *E. zollingeri* because it occurs in shaded forest understorey with a paucity of

35   pollinators.

36

37   **Keywords** autogamy, breeding system, geographic variation, gynomonoecy, mixed mating,

38   mycoheterotrophy, outcrossing, reproductive assurance, self-pollination

39

Introduction

41   The Orchidaceae are one of the largest and most morphologically diverse families of land plants,

42   and include more than 28,000 species classified into ~760 genera (*Christenhusz & Byng, 2016*).

43   Variations in its floral characteristics and their effects on reproductive success have long

44   intrigued botanists since the time of Darwin (*Inoue, 1986*; *Nilsson, 1988*; *Sletvold & Gren*,

45   *2011*). However, it is noteworthy that while the vast majority of orchid species produce only

46   hermaphroditic flowers (i.e., flowers that possess both male and female reproductive organs;

47   *Pannell, 2009*), a variety of sexual polymorphisms (the co-occurrence of morphologically

48   distinct sex phenotypes within the same species) can be found in flowering plants as a whole

49   (*Barrett, 2010*).

50   In fact, almost all orchid species exhibit an extreme case of hermaphroditism, owing to the

51   fusion of male and female organs into a gynostemium (*Rudall & Bateman, 2002*). Exceptions to

52   this rule have only been reported from the subtribes, Catasetinae and Satyriinae (*Pannell, 2009*;

53   *Romero & Nelson, 1986*; *Huang et al., 2009*). More specifically, within Catasetinae, the

54   members of *Catasetum* Rich. ex Kunth and *Cycnoches* Lindl. typically exhibit dioecy (i.e.,

55   unisexual individuals). In the dioecious *Catasetum*, male flowers forcibly attach a large

56   pollinarium onto euglossine bees, and the bees subsequently avoid flowers with the same

57   appearance (*Romero & Nelson, 1986*). Therefore, *Catasetum* populations are sexually dimorphic,

58   and the agitated pollinators bearing their pollinia move away from the male flowers to the

59   morphologically different female flowers (*Romero & Nelson, 1986*). In addition, within

60   Satyriinae, *Satyrium ciliatum* Lindl. has been reported to produce both hermaphroditic and

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61   female individuals (i.e., gynodioecy; *Huang et al.*, *2009*). Female individuals of *S. ciliatum* can

62   avoid pollen limitation for seed production and can be maintained in populations that experience

63   high levels of pollen limitation, because females, via facultative parthenogenesis, can produce

64   more seeds than do hermaphrodites (*Huang et al.*, *2009*). These observations helped elucidate the

65   unusual maintenance of gender dimorphism in orchids (*Pannell*, *2009*).

66   Here, I report an additional orchidaceous example whose flowers are not always

67   hermaphroditic. In a Japanese population of *Eulophia zollingeri* (Rchb.f.) J.J.Sm, a widespread

68   Asian and Oceanian orchid, some flowers possess both the anther (i.e., anther cap and pollinaria)

69   and stigma, whereas others possess only the stigma (Fig. 1). It is unlikely that the absence of

70   anther cap and pollinaria is the result of removal by floral visitors, because careful dissection

71   revealed that they were already absent before anthesis. Such a sexual system, in which plants

72   have both female and hermaphroditic flowers co-occurring within the same plants, is called

73   gynomonoecy. Compared with andromonoecy (male and hermaphroditic flowers within one

74   plant) and monoecy (separate male and female flowers on the same plant), gynomonoecy

75   remains a poorly studied sexual system, even though it occurs in 2.8–4.7% of flowering plants in

76   at least 15 plant families (*Lu & Huang*, *2006*; *Yampolsky & Yampolsky*, *1922*).

77   In fact, while several hypotheses have been proposed, the adaptive significance of

78   gynomonoecy remains largely unknown. First, the presence of the two flower types may permit

79   flexible allocation of resources to female and male reproductive functions in response to

80   environmental conditions (*Charnov & Bull*, *1977*; *Lloyd*, *1979*). Second, female flowers may

81   promote outcrossing more than hermaphroditic flowers (*Marshall & Abbott*, *1984*). Third, due to

82   the lack of evidence for the above two hypotheses, it has been proposed that female flowers may

83   boost attractiveness to pollinators (*Bertin & Kerwin*, *1998*). Finally, female flowers may be

84   favored because hermaphroditic flowers, which are more attractive in gynomonoecious plants,

85   are more susceptible to florivory (*Bertin, Connors & Kleinman*, *2010*; *Zhang, Xie & Du*, *2012*).

86   Meanwhile, current understanding of the adaptive advantages of gynomonoecy is largely limited

87   to the Asteraceae and to a few species that have been investigated in other families (*Davis &*

88   *Delph*, *2005*; *Mamut et al.*, *2014*).

89   Therefore, I investigated the reproductive biology of a Japanese *E. zollingeri* population,

90   which potentially represents the first documentation of gynomonoecy within Orchidaceae, to

91   understand the ecological significance of the different reproductive strategies of the two floral

92   morphs within one individual. Autonomous self-pollination has been suggested to be favorable

93   for mycoheterotrophic plants, as they are restricted to dark shaded forest understorey with a

94   paucity of pollinators (*Leake, 1994*; *Zhou et al., 2012*; *Suetsugu, 2013a, 2015*). However, the

95   Red Queen hypothesis suggests that sexual reproduction is important in a coevolutionary arms

96   race between a parasite and host (*Ladle, 1992*), such as between mycoheterotrophic plants and

97   fungal hosts. Intriguingly, Zhang *et al. (2014)* revealed Chinese populations of *E. zollingeri* to be

98   hermaphroditic, non-rewarding, self-compatible, and dependent on the halictid bee *Nomia*

99   *viridicinctula* Cockerell for pollination through food deception. Although those Chinese

100   populations consequently experienced strong pollinator limitation, especially in forest

101   understorey populations *(Zhang et al., 2014)*, my preliminary investigation revealed that

102   Japanese populations consistently exhibit high fruit set even under the shaded forest understorey.

103   Therefore, it is possible that the Japanese hermaphroditic flowers are capable of autonomous

104   selfing, providing reproductive assurance, while female flowers enhance outcrossing

105   However, it should also be noted that the "female" *E. zollingeri* flowers can be sterile

106   without not only the male but also the female reproductive function. In fact, in *Catasetum*

107   species that produce male and female flowers, intermediate flowers, which are sterile, have also

108   been found (*Romero, 1992*). Therefore, I first investigated whether the "female" flowers of *E.*

109   *zollingeri* possess female reproductive functions. After that, I conducted additional pollination

110   experiments, investigations of floral morphology, and observations of floral visitors to determine

111   the potential for reproductive assurance provided by autonomous selfing in female flowers and

112   outcrossing via pollinator visitation in both hermaphroditic and female flowers.

113

Materials & Methods

115   *Eulophia zollingeri* is a mycoheterotrophic orchid distributed from India and Southeast Asia to

116   New Guinea and Australia (*Ogura-Tsujita & Yukawa, 2008*; *Suetsugu & Mita, 2019*; *Suetsugu,*

117   *Matsubayashi & Tayasu*, 2020). The behavior of floral visitors in Miyazaki City, Miyazaki

118   Prefecture, Japan, was monitored during the peak flowering period (early to mid-July), in 2016

119   and 2017. Direct observations were made for ca. 30 h in total, during the peak of diurnal insect

120   activity (09:00–17:00). The behavior of potential visitors was observed by walking around the

121   study site, sitting next to flower patches, or hiding in the vegetation near flower clusters (within

122   1–2 m). In addition, artificial cross-pollination was performed in the same population in July 2016,

123   by transferring pollinaria from different individuals to the stigmas of female flowers (five
124   inflorescences, 10 flowers).

125   After confirming with the cross-pollination experiment that both hermaphroditic and female
126   flowers produce fruits, additional pollination experiments were conducted in early July 2017.
127   Flowers were either (i) manually cross-pollinated by transferring pollinaria to the stigmas of
128   different individuals (five inflorescences, 10 each of female and hermaphroditic flowers); (ii)
129   manually geitonogamous-pollinated by transferring pollinaria to the stigmas of the different
130   flowers within the same individuals for female flowers and of the same flowers for hermaphroditic
131   flowers (five inflorescences, 10 each of female and hermaphroditic flowers); (iii) enclosed in mesh
132   bags to exclude floral visitors and test for autonomous self-pollination (five inflorescences, 10
133   hermaphroditic flowers); or (iv) left unmanipulated, in order to monitor fruit set under natural
134   conditions (seven inflorescences, 19 female flowers and 21 hermaphroditic flowers). In addition,
135   the relative position of female and hermaphroditic flowers on the racemes was determined in 12
136   inflorescences. Furthermore, to compare flower size between female and hermaphroditic flowers
137   in gynomonoecious individuals, we measured the length of the dorsal sepal, lateral sepal, lateral
138   petal, and lip of 10 plants using digital calipers to 0.1 mm in early July 2017. Finally, the
139   distribution of female flowers was checked in 12 inflorescences in early July 2017. After dividing
140   each inflorescence into distal and proximal halves, the data were tested using the Mann–
141   Whitney U-test to investigate whether female flowers tended to be in the distal or basal part of the
142   inflorescence.

143   Three to four months after manual pollination, all the mature but non-dehisced fruits
144   capsules were collected. After the fruits were silica-dried, I weighed the total mass of dry seeds
145   freed from each capsule to the nearest 0.1 mg. All the seeds from each plant were then mixed, and
146   100 randomly selected seeds from each plant were examined under a dissecting microscope to
147   determine presence of an embryo. The effects of pollination treatment on fruit set were tested using
148   Fisher's exact test. In addition, after confirming that the datasets were normally distributed using
149   Levene's test, the effects of pollination treatment on the seed mass, and the proportion of seeds
150   with an embryo were tested using ANOVA, followed by Fisher's multiple comparisons test.

151

152   **Results**

153   Despite conducting ca. 30 h of field observations, few insects were observed visiting the *E.*

154   *zollingeri* flowers. Several dipteran visitors, such as the agromyzid fly *Japanagromyza*

155   *tokunagai*, occasionally landed on the flowers. However, none of these visitors were observed to

156   remove or deposit pollinaria. The length of the dorsal sepal, lateral sepal, lateral petal, and lip

157   were not significantly different between female and hermaphroditic flowers (Table 1). In

158   addition, there are marginally significant differences in the number of female flowers between

159   the distal half (1.2 ± 1.1; mean ± SD) and the proximal half (3.3 ± 3.4; $P = 0.06$) of the

160   inflorescence.

161   More than half (6/10) of the female flowers subsequently developed fruit capsules that

162   contained seeds with an embryo through artificial cross-pollination in 2016, thereby

163   demonstrating their female function and confirming the gynomonoecy of the species. The results

164   are stable at least in the investigated site, because similar results were obtained in 2017 (Table 2).

165   The detailed pollination experiments showed that the bagged female flowers failed to develop

166   fruits autonomously, excluding the possibility of agamospermy, while comparable fruit set ratio

167   was also obtained in open, bagged, manual geitonogamous and allogamous hermaphroditic

168   flowers. Therefore, the hermaphroditic flowers are capable of outbreeding, but self-compatible

169   and not pollinator-limited for fruit set under natural condition (Table 2). The seed mass did not

170   vary significantly with pollination treatment (ANOVA $F_{6, 37} = 1.17$, $P = 0.34$), while the

171   proportion of seeds with an embryo differed significantly among pollination treatment (ANOVA

172    $F_{6, 37} = 2.43$, $P = 0.04$). In general, the pollination experiments indicated that outcrossing tended

173   to increase both seed mass and the number of seeds with embryo, suggesting a negative impact

174   of self-pollination, although the differences were not always significant (Table 2).

175   The observation of floral morphology confirmed that most of the hermaphroditic flowers

176   possessed an effective self-pollination system, in which the rostellum was poorly developed,

177   allowing contact between the stigma and pollinaria (Fig. 2B), whereas the others had functional

178   rostella and were therefore unlikely to be autogamous (Fig. 2D). The female flowers had a

179   column with neither a rostellum nor anther cap and pollinaria (Fig. 2F).

180

181   **Discussion**

182   Most orchid species exhibit an extreme case of hermaphroditism, owing to the fusion of male

183   and female organs into a gynostemium. Here I showed that a Japanese population of *Eulophia*

184 *zollingeri* develops both female and hermaphroditic flowers co-occurring within the same

185 inflorescence (i.e., gynomonoecy), while *Catasetum* and *Cycnoches* typically produces unisexual

186 individuals (i.e., dioecy, *Romero & Nelson, 1986*), and *Satyrium ciliatum* produces both

187 hermaphroditic and female individuals (i.e., gynodioecy; *Huang et al., 2009*). Therefore, the

188 present study represents the first example of gynomonoecy within the Orchidaceae. However, it

189 should be noted that gynomonoecy must not be considered a universal strategy within the species

190 as a whole, since it was not reported in the Chinese study (*Zhang et al. 2014*). In this sense, it

191 differs from the fixed systems in *Catasetum, Cycnoches,* and *Satyrium.* The hermaphroditic

192 flowers of a Japanese *E. zollingeri* population often possess an effective self-pollination system,

193 while the female flowers without agamospermy can improve the probability of outcrossing (but

194 selfing may still occur via geitonogamous pollinations). While female flowers are generally

195 smaller than hermaphroditic flowers in other gynomonoecious species (reviewed by *Mamut et*

196 *al., 2014*), the size of floral parts did not differ significantly between female and hermaphroditic

197 flowers of *E. zollingeri*. In addition, female flowers tend to be on the lower part of the

198 inflorescence, suggesting that production of female flowers is not a result of resource

199 competition. In summary, the system observed in *E. zollingeri* is consistent with the outcrossing-

200 benefit hypothesis for gynomonoecy (*Mamut et al., 2014*).

201   Many models predict that plants evolve toward either complete self-fertilization or

202 complete outcrossing (*Charlesworth & Charlesworth, 1990*). However, it seems that mixed-

203 mating systems are more common in nature (*Vogler & Kalisz, 2001*; *Whitehead et al., 2018*),

204 possibly because mixed mating can reduce the probability of inbreeding depression via

205 outcrossing, while still providing reproductive assurance via selfing (*Goodwillie & Weber*,

206 *2018*). As such, mixed mating systems are often referred to as "best-of-both-worlds" mating

207 systems (*Davis & Delph, 2005*; *Goodwillie & Weber, 2018*). Mixed mating can be accomplished

208 by delayed selfing, occurring after all other opportunities for outcrossing have been missed,

209 because it provides reproductive assurance without limiting outcrossing opportunities. In

210 addition, mixed mating can also occur in species that produce two flower types within the same

211 plant. Gynomonoecy is one of the systems involving two flower types that allows for mixed

212 mating. Indeed, in the *E. zollingeri* populations investigated here, hermaphroditic flowers

213 conferred reproductive assurance under pollinator-limited conditions, whereas female flowers,

214   despite their susceptibility to pollen limitation, can facilitate outcrossing, because of the lack of

215   autonomous selfing (Table 2).

216   It is possible that geitonogamy reduces the possibility of outcrossing in female flowers. In

217   *E. zollingeri*, though, the level of geitonogamy will be low, because only a few flowers on each

218   plant are open at one time. In particular, the risk of geitonogamy is probably negligible in

219   nectarless *E. zollingeri*, given that pollinators are likely to quickly leave inflorescences in food-

220   deceptive plants (*Zhang et al.*, *2014, 2019; Suetsugu et al., 2015*). Indeed, the avoidance of

221   geitonogamy has been hypothesized as a driving force for the evolution of food deceptive

222   pollination in plants (*Johnson, Peter & Ågren, 2004*). Moreover, it is noteworthy that female

223   flowers tended to be on the lower part of the inflorescence, given that *E. zollingeri* were

224   exclusively pollinated by the halictid bee *Nomia viridicinctula* in China (*Zhang et al., 2014*) and

225   that bees usually visit bottom flowers first and move upwards within an inflorescence (e.g. *Iwata

226   et al., 2012*). In fact, several studies have shown that pollinator behaviors lead to directional

227   pollen flow within inflorescences and influence floral sex allocation (*Brunet & Charlesworth*,

228   *1995*). The first flowers visited will receive more pollen grains from other plants, while the last

229   flowers visited before pollinators leave the inflorescence tend to receive geitonogamous

230   pollination but successfully export pollen grains to other plants (*Kudo, Maeda & Narita, 2001*).

231   Therefore, it has been predicted that female-biased allocation to lower flowers and male-biased

232   allocation to those in upper positions occurs in bee-pollinated plants (*Kudo, Maeda & Narita*,

233   *2001*). The variations in floral sex allocation within *E. zollingeri* are consistent with the theory

234   and are probably effective for lowering the risk of geitonogamy.

235   The advantages of outcrossing and, consequently, producing female flowers can be

236   somewhat influenced by the degree of inbreeding depression and pollinator availability

237   (*Smithson, 2006*). In *E. zollingeri*, pollinator-mediated fruit set was arguably low, at least in the

238   investigated population, given that (i) direct pollinator observation was unsuccessful and (ii)

239   pollination experiments showed that natural pollination in female flowers was recorded only in

240   one flower. Nonetheless, a small degree of outcrossing can result in a rapid decline in linkage

241   disequilibrium across the genome and can be sufficient to overcome negative effects such as the

242   accumulation of deleterious mutations and the slowdown in adaptation rate (*Culley & Klooster*,

243   *2007*). In addition, although the differences were not obvious (*Tremblay et al. 2005*), both

244   artificial allogamous pollination and natural pollination in a female flower tended to increase

245 seed mass and the proportion of seed with an embryo in *E. zollingeri* (Table 2), probably

246 providing some support for the negative effect of autonomous selfing. It should be noted that,

247 while seed mass and presence of an embryo was measured as the indicator of inbreeding

248 depression, it can even under-estimate the level of inbreeding depression. Inbreeding depression

249 might be more prominent during later stages such as seed germination or seedling growth

250 (*Smithson*, *2006*). This possibility warrants further investigation.

251   The outcrossing opportunity might be particularly important in mycoheterotrophic plants

252 exploiting their mycorrhizal partners (*Suetsugu et al.*, *2017*), given that they usually occur in

253 shaded understorey habitats with a paucity of pollinators, and that the Red Queen hypothesis

254 argues that outcrossing is maintained by antagonistic interactions between a host and a parasite

255 (*Ladle*, *1992*; *Gibson & Fuentes*, *2015*). Because mycoheterotrophic plants occur mainly in

256 pollinator-hostile shaded understorey habitats, they tend to experience strong pollinator limitation,

257 unless they possess autonomous selfing ability (*Klooster & Culley*, *2009*; *Hentrich, Kaiser &*

258 *Gottsberger*, *2010*; *Suetsugu*, *2013a, 2015*). In fact, the Chinese populations without autogamous

259 ability exhibited a significant difference in fruit-set between forest edge and forest populations

260 (*Zhang et al.*, *2014*). Therefore, pollination limitation due to its mycoheterotrophic habit could

261 be a driving force in the autonomous self-pollination in *E. zollingeri.* Consequently, most studies

262 highlighted the importance of autonomous self-pollination in mycoheterotrophic plants (*Leake*,

263 *1994*; *Zhou et al.*, *2012*; *Suetsugu*, *2013a, 2015*). However, several recent studies have shown that

264 mixed mating systems such as outcrossing pollinators with delayed self-pollination occur in

265 mycoheterotrophic species belonging to Ericaceae and Gentianaceae, which evolved

266 mycoheterotrophy independently from *E. zollingeri* (*Klooster and Culley, 2009; Hentrich et al.,*

267 *2010*). The mixed mating systems, including gynomonoecy, might be more common and important

268 in mycoheterotrophic plants than previously thought.

269   Overall, it can be concluded that the Japanese population of *E. zollingeri* studied here

270 preserve reproductive assurance by producing autonomously selfing hermaphroditic flowers and

271 still maintain the potential benefit of producing outcrossed offspring by developing female

272 flowers. In addition, while I did not conduct the pollination experiments, several other Japanese

273 populations such as Okinawa ones exhibit very similar floral morphology *(Fig. S1)*, suggesting

274 that the strategy might be widespread at least in Japan. However, intriguingly, the Chinese

275 populations appear to develop hermaphroditic flowers that are completely dependent on bee

276 pollinators *(Zhang et al., 2014)*. In fact, it is well-known that plant mating systems often vary

277 widely among populations *(Suetsugu, 2013b; Whitehead et al., 2018)*. Variations in mating

278 systems between populations usually reflect the influence of ecological factors such as the

279 availability and abundance of suitable pollinator *(Suetsugu, 2013b; Schouppe et al., 2017;*

280 *Whitehead et al., 2018)*. Therefore, it is worth clarifying how common gynomonoecy with

281 autonomous selfing hermaphroditic flowers and putatively outcrossing female flowers, is across

282 the distribution range and whether the strategy is be more prevalent where its effective pollinator

283 is less abundant.

284   It is also notable that current understanding of the adaptive advantages of gynomonoecy is

285 largely limited to the Asteraceae *(Marshall & Abbott, 1984; Bertin & Kerwin, 1998; Bertin,*

286 *Connors & Kleinman, 2010; Zhang, Xie & Du, 2012)*. The outcrossing hypothesis of

287 gynomonoecy has been questioned in many asteraceous taxa, given that most Asteraceae species

288 are self-incompatible *(Bertin & Kerwin, 1998)*. However, it has been shown that hermaphroditic

289 flowers promote seed quantity in that they are more attractive to pollinators and/or are capable of

290 autonomous selfing, while female flowers compensate for loss of male function through

291 outcrossing in non-asteraceous taxa [i.e., *Silene noctiflora* (Caryophyllaceae) and *Eremurus*

292 *anisopterus* (Xanthorrhoeaceae)] *(Davis & Delph, 2005; Mamut et al., 2014)*. Taken together

293 with these recent finding, I suggest that the ability of female flowers to reduce geitonogamy and

294 enhance outcrossing may be widespread in gynomonoecious plants. However, it should be noted

295 that although many orchids are (at least partially) parasitic on their mycorrhizal fungi and exhibit

296 strong pollinator-limitation *(Leake, 1994)*, gynomonoecy is not prevalent within the orchid

297 family as a whole. Given that (i) hypotheses regarding the adaptive significance of gynomonoecy

298 are not mutually exclusive and (ii) the seed-feeding fly *Japanagromyza tokunagai* have probably

299 substantial negative impact on the reproduction of *E. zollingeri* *(Suetsugu & Mita, 2019)*,

300 benefits other than outcrossing, such as herbivory reduction, could also have contributed to the

301 evolution of gynomonoecy. Therefore, further investigation is needed to elucidate the potentially

302 diverse adaptive significance, disadvantages, and developmental constraints of gynomonoecy.

303

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308

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428

429   **Figure legends**

430

431   **Figure 1 *Eulophia zollingeri* in its natural habitat.** (A) Inflorescence. (B) Hermaphroditic

432   flower. (C) Female flower.

433

434   **Figure 2 Column morphology of *Eulophia zollingeri* flowers.** (A, B) Column with a

435   degenerate rostellum, which facilitates autogamy. (C, D) Column with a well-developed

436   rostellum, which prevents autogamy. (E, F) Column with neither a rostellum nor anther cap and

437   pollinaria. AC, anther cap; RS, rostellum; PO, pollinaria; ST, stigma.

Figure 1

Eulophia zollingeri in its natural habitat

Eulophia zollingeri in its natural habitat. (A) Inflorescence. (B) Hermaphroditic flower.
(C) Female flower.

Figure 2

Column morphology of *Eulophia zollingeri* flowers.

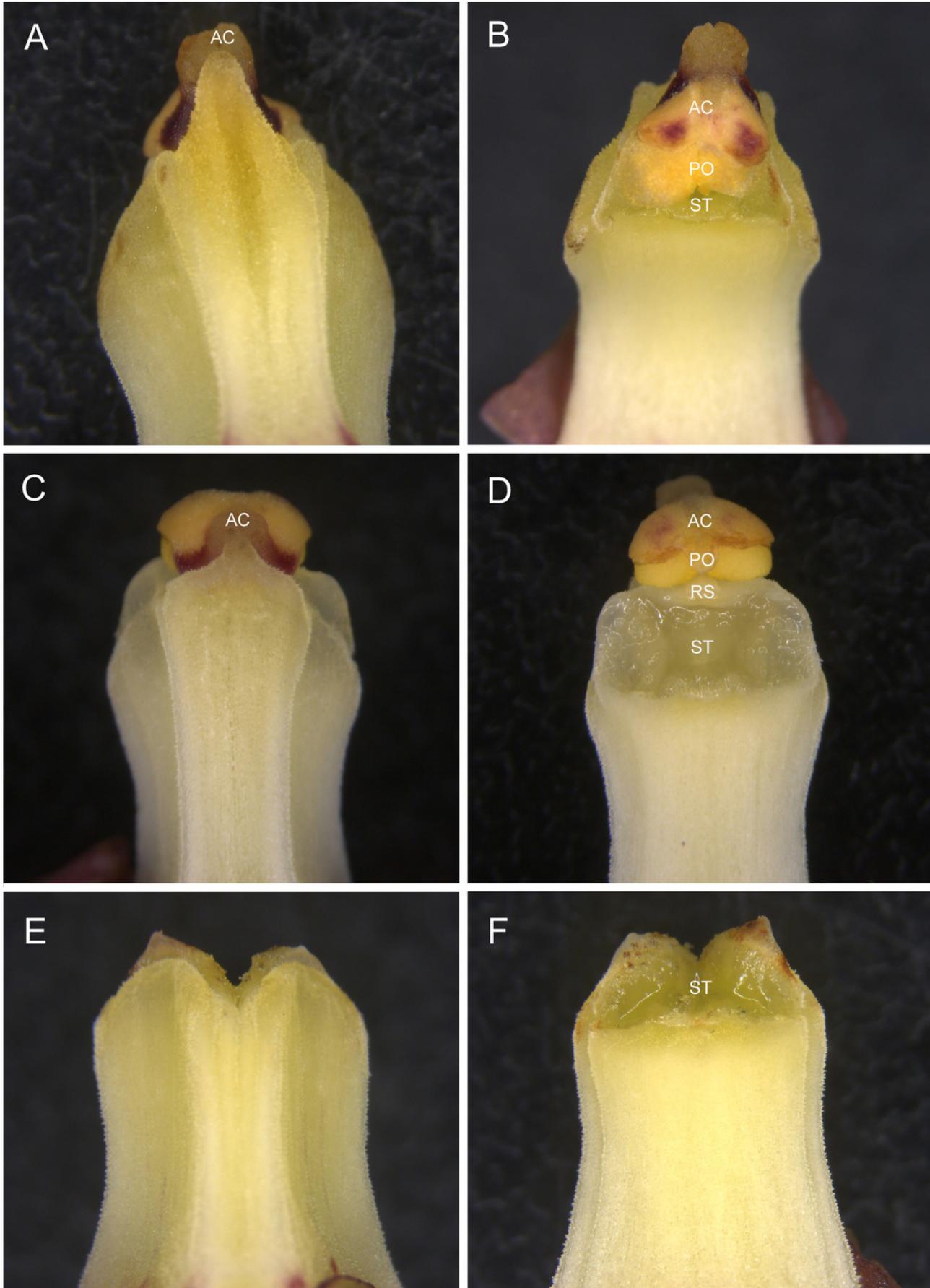
Column morphology of *Eulophia zollingeri* flowers. (A, B) Column with a degenerate rostellum, which facilitates autogamy. (C, D) Column with a well-developed rostellum, which prevents autogamy. (E, F) Column with neither a rostellum nor anther cap and pollinaria. AC, anther cap; RS, rostellum; PO, pollinaria, ST, stigma.

Comparison of the length of the dorsal sepal, lateral sepal, lateral petal, and lip, between hermaphroditic and female flowers in *Eulophia zollingeri*

Table 1. Comparison of the length of the dorsal sepal, lateral sepal, lateral petal, and lip, between hermaphroditic and female flowers in *Eulophia zollingeri*.

Flower type	Dorsal sepal (mm)	Lateral sepal (mm)	Lateral petal (mm)	Lip (mm)
Hermaphroditic	23.5 ± 1.8	25.6 ± 2.1	17.8 ± 1.5	19.7 ± 0.7
Female	24.8 ± 1.6	26.2 ± 1.8	18.2 ± 1.3	20.0 ± 0.9

The lengths are expressed by mean ± SD (mm).

Effect of pollination treatment on fruit set, seed mass, and proportion of seeds with embryo in *Eulophia zollingeri*

Table 2. Effect of pollination treatment on fruit set, seed mass, and proportion of seeds with an embryo in *Eulophia zollingeri*.

Flower type	Treatment	Manual allogamy	Manual geitonogamy	Autonomous autogamy	Open
Hermaphroditic	Fruit set (%)	70.0[a]	70.0[a]	50.0[a]	57.1[a]
	Seed mass	31.6 ± 11.6	24.1 ± 11.4	20.8 ± 9.3	24.9 ± 8.6
	Seeds with embryo	82.4 ± 4.2[ac]	79.0 ± 4.2[abc]	77.2 ± 2.9[bc]	79.2 ± 3.6[c]
Female	Fruit set (%)	60.0[a]	60.0[a]	0.0	5.3[b]
	Seed mass	30.2 ± 14.7	25.7 ± 11.0	–	44.9
	Seeds with embryo	83.3 ± 4.4[a]	77.3 ± 2.9[bc]	–	82.0[abc]

Different superscript letters indicate significant differences ($P < 0.05$) between treatment groups. Both seed mass and seeds with an embryo are expressed by mean ± SD, whenever the sample size is more than > 1.