1   When to be a male? Role of resource-limitation and pollinators in determining gender in an

2   andromonoecious spiderwort.

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26   **Abstract**

27   The evolution and maintenance of sexual systems in plants is often driven by resource

28   allocation and pollinator preferences. Gender or sex in plants, therefore, represents a

29   physiological, functional, and behavioral response of a plant to its environment. Annual,

30   entomophilous plants have three major constraints towards optimal reproduction: 1) nutrient

31   resources obtained from the environment, 2) nutrient resources allocated towards

32   reproduction, i.e., fruits vs. flowers, and 3) pollinator visitations. We know very little about

33   the role of nutrient resources and pollinators in driving gender expression in flowers in a

34   natural environment.

35   Andromonoecy is a sexual system where the plant bears both staminate and

36   hermaphrodite flowers on the same inflorescence and it occurs in ~2% of flowering plants.

37   Although it has been shown that andromonoecy has evolved multiple times within the

38   angiosperms, our understanding of the underlying mechanisms of what determines gender

39   expression in nature is limited. The optimal resource allocation hypothesis, one of the most

40   widely accepted hypotheses which describes evolution of andromonoecy, suggests that under

41   nutrient constraints, plants will produce more male flowers since they require lesser energy

42   investment than the more expensive hermaphrodite flowers. We test this hypothesis in an

43   andromonoecious plant *Murdannia simplex* (Commelinaceae) by measuring gender

44   distributions in a large natural population and contrasting the gender distributions between

45   two different resource conditions (stream population vs. plateau population). We also carried

46   out choice experiments to quantify if pollinators showed any preference towards a gender.

47   We found that in *M. simplex*, production of hermaphrodite flowers is resource-

48   dependent and under resource constraints not only fewer numbers of flowers were produced

49   but also there were a higher number of male flowers. We failed to observe pollinator

50   preference towards either gender but *Amegilla spp. (Zonamegilla)* and *Apis cerana* showed

51 frequency-dependent visitation which suggests that selection on inflorescence size may be an

52 indirect form of selection pressure exerted by pollinators resulting in gender-bias in the

53 plants. Thus, we conclude that environmentally driven resource constraints play a bigger role

54 in driving floral gender expression in *Murdannia* over direct pollinator-driven constraints.

55 **Keywords**

56 Andromonoecy, Commelinaceae, floral gender, male, hermaphrodite, *Murdannia simplex*,

57 resource limitation, pollinator preference, Western Ghats

58 **Introduction**

59 Andromonoecy refers to the sexual system where both staminate (male) flowers and perfect

60 (hermaphrodite) flowers are present on the same plant (Yampolsky and Yampolsky 1922,

61 Bertin 1982a, Miller and Diggle 2003, Vallejo-Marín and Rausher 2007a, 2007b). In

62 andromonoecious plants, the hermaphrodite flower fulfills the female function in the absence

63 of a true 'female' flower, and it has been suggested that andromonoecy probably evolved

64 from hermaphroditism by the loss of female reproductive structure (Lloyd 1980, Bertin

65 1982a). One of the most widely accepted hypotheses on the evolution of andromonoecy is the

66 optimal resource allocation hypothesis which suggests that under resource limitation, male

67 flowers will be produced instead of hermaphrodite flowers. This hypothesis rests upon the

68 premise that male flowers are energetically cheaper to produce because reproductive

69 allocation towards them ends with pollen production (Anderson and Symon 1989, Narbona et

70 al. 2002, Verdú et al. 2007), while females are expensive because fruit development and seed

71 germination are energy-intensive physiological processes (Bertin 1982a, Kaul et al. 2002,

72 Obeso 2002, Verdú et al. 2007).

73   Examples of reallocation of resources to the cheaper sex (i.e. male bias) have been

74 shown in several plant species where staminate flowers are produced when nutrient resources

75 become scarce due to its utilization by the costlier gynoecia towards fruit production (Bertin

76   1982a, 1982b, May and Eugene Spears Jr 1988, Miller and Diggle 2003, Venkatesan 2017).

77   A well-studied species is *Solanum hirtum* (Solanaceae) in which it has been shown that as a

78   result of successful pollination, as the ovary develops, resources are relocated within an

79   inflorescence to the male flowers, thus influencing gender expression and distribution of

80   gender in successive flowers (Diggle 1993, Miller and Diggle 2003). Another example comes

81   from *Raphanus raphanistrum*, where Stanton et al. (1987) showed that in plants which were

82   heavily pollinated, the number of ovules per flower decreased and in contrast, unpollinated

83   plants did not show any significant decrease in number of ovules. This suggests that plants

84   can modify their reproductive output to adjust for the resource limitation faced by them.

85   In plants, resource limitation or nutrient limitation that can affect floral gender

86   expression can be identified as: a) environmental nutrient limitation (Charnov and Bull 1977,

87   Primack and Lloyd 1980a, Stephenson 1981, Bertin 1982a, Solomon 1985, Diggle 1993),

88   and/or b) within-plant nutrient limitation (Stephenson 1981, May and Eugene Spears Jr 1988,

89   Diggle 1993, 1995, Miller and Diggle 2003, Ortiz et al. 2003, Vallejo-Marín and Rausher

90   2007a). In *Aesculus californica*, *A. pavia*, *Leptospermum scoparium* and *Passiflora*

91   *incarnata*, phenotypic gender expression (hermaphroditic inflorescences or hermaphroditic

92   flowers) has been shown to vary as a response to environmental conditions or status of the

93   nutrient resources, especially during fruit development when resources are limited (Benseler

94   1975, Primack and Lloyd 1980b, May and Eugene Spears Jr 1988). Further, it has been

95   shown that the amount of resources that a plant acquires in the proximal and distal ends of the

96   inflorescence can also vary. The proximal/ basal end of the inflorescence has more resources

97   than its distal end, which results in more hermaphrodite flowers to be present at basal ranks

98   of an inflorescence, while male flowers are relegated to the distal end (Faden 1991, Diggle

99   1994, 1995, 1997, Lewis and Gibbs 1999, Ashman and Hitchens 2000, Miller and Diggle

100   2003, Kaul and Koul 2008).

101   Bateman's principle (Bateman 1948, Wilson et al. 1994) asserts that- "Fitness gain

102   through male function is limited primarily by mating opportunity, while fitness gain through

103   female function is limited primarily by resource availability for offspring production". To

104   explain the role of male flowers in andromonoecious plants, two, not mutually exclusive

105   hypotheses have been proposed (Zhang and Tan 2009): i) Pollen donor hypothesis

106   approaches the problem from the perspective of male reproductive success. It predicts that the

107   wastage of nutrient resources by investment in the female reproductive structure is reduced

108   by the production of only male flowers, in a resource-limited condition. ii) Pollinator attractor

109   hypothesis is based on female reproductive success. It predicts that male flowers increase the

110   floral display and attract more pollinators at a lower cost and thereby increase the stigmatic

111   load of the hermaphrodite flowers, which potentially increases female reproductive success at

112   lower resource investment. The sex allocation theory predicts that by attracting pollinators

113   which promote outcrossing, floral attractiveness (e.g., large petals, nectar availability) gains

114   more fitness through the male function than female function (Elle and Meagher 2000).

115   Although male success should be correlated with mating opportunities, one or a few visits by

116   pollinators are known to be adequate to fertilize all ovules. Therefore, it is not expected that

117   female fitness would be strongly affected by floral attractiveness. Thus, in sexually

118   reproducing plants, investment in the production of flowers is very important since this can

119   directly translate into reproductive success, and hence reproductive fitness. In entomophilous

120   plants, pollinator dependence is often driven by floral characteristics, and for successful

121   pollination, the gender of the flowers and associated floral characters may be very important

122   (Armbruster 2001, Fenster et al. 2004). In many hermaphroditic plant systems, it has been

123   shown that several characteristics of an inflorescence such as number of flowers, size of

124   flowers, and reward of flowers play a critical role in attracting pollinators to the plant (Ortiz

125   et al. 2003, Christopher et al. 2019). While the role of pollinators is very well known in

126   hermaphroditic systems, the perception of gender, pollinator preference, and reproductive

127   output in andromonoecious systems are not well studied (see Diggle 1993, Ashman et al.

128   2000, Ashman and Morgan 2004).

129   Previous studies in sexually dimorphic plants have shown that pollinators typically

130   show a preference for the functionally male flowers over the conspecific functional female

131   flowers owing to different floral traits like petal length (Ashman et al. 2000), levels of pollen

132   production (Bierzychudek 1987, Eckhart 1991) and/or floral scents (Ashman et al. 2005),

133   where these preferences are learned through foraging experience (Cresswell and Galen 1991).

134   Zhang and Tan (Zhang and Tan 2009) were the first to examine the function of male flowers

135   in the andromonoecious *Capparis spinosa* where they showed that the pollinators did not

136   discriminate between the floral gender morphs, even when they were morphologically

137   distinct. However, similar studies investigating gender-specific pollinator preferences and

138   more specifically gender-specific pollinator preferences in sexually dimorphic plants that

139   have near-identical morphological features are not known.

140   In this study, we explore the effect of nutrient resources as well as pollinator

141   preference in driving gender expression in a wild population of the andromonoecious

142   *Murdannia simplex* (Commelinaceae, Spiderworts). The pantropical plant family

143   Commelinaceae (Spiderworts) is known to have several genera that display andromonoecy.

144   Despite the dominance of andromonoecy in this family, to date, most pollination studies

145   within this family have focused only on identifying pollinators (Kaul et al. 2002, Williams

146   and Walker 2003, Oziegbe et al. 2013, Sigrist and Sazima 2015), and only one study has

147   explored the role of floral guides in pollinator attraction (Ushimaru et al. 2007). Based on

148   predictions from the optimal resource allocation hypothesis and pollinator attractor

149   hypothesis we address the following questions:

150   A) Does the proportion of staminate and perfect flowers in a population represent resource

151   limitation?

152   B) Do pollinators show preference to any one of the genders?

153   C) Do staminate flowers improve floral display to maintain visitation rate and function as a

154   cheaper source of outcrossing pollen?

155   **Materials and methods**

156   *Murdannia simplex* (Vahl) Brenan (Commelinaceae) is a mass-flowering, sub-erect, annual,

157   andromonoecious herb which is widely distributed in moist deciduous forests and grasslands

158   of the Western Ghats of India (Nandikar and Gurav 2015; Fig. 1a). The plant is seasonal, and

159   the shoot emerges once the monsoonal rains begin, and the flowering lasts from September to

160   November (Nandikar and Gurav 2015). The plant is 40-65 cm tall and bears a cymose

161   inflorescence which is a panicle. The inflorescence bears 5-20 purple-colored, three-petaled

162   male and hermaphrodite flowers (andromonoecy), and each flower measures ~15-25 mm in

163   diameter. The hermaphrodite flowers are characterized by the presence of a lateral pistil, with

164   two upper fertile stamens, curved downwards and a third lower sterile stamen. Filaments

165   have long purple, bearded hair. The flower also bears three trilobed sterile staminodes with

166   glabrous filaments. The male flowers are characterized only by the lack of a pistil (Fig. 1b -

167   f). The flowers are defined by a short floral anthesis time, from 12:30 hrs. to 16:00 hrs., with

168   the principal pollinator reward being pollen grains only (Faden 2000, Nandikar and Gurav

169   2015).

170   *Study site*

171   Western Ghats is one of the four biodiversity 'hotspots' in India. The northern part of

172   Western Ghats is famous for high elevation laterite plateaus and slopes, one of them being

173   Kaas plateau in Maharashtra (17° 43′ 12″ N, 73° 49′ 22″ E), which is located at an altitude of

174   ~1225 m above sea level (Fig. 1a). Kaas plateau has an area of ~10 km^2 and is situated

175 northwest, about 25 km away from the city of Satara in the western state of Maharashtra. It

176 was declared as a UNESCO World Heritage site in 2012 due to its highly endemic floral

177 diversity, which is most abundant in the months of August to November.

178   Capped with red lateritic crusts, the upper Kaas Plateau provides arid habitats except

179 during the monsoon season (Fig. 1a). It receives over 2500 mm of rain every year, mainly

180 during the monsoon months (June to September), and the daily mean temperatures are over

181 22°C (Bhattarai et al. 2012). Floral diversity on the Kaas plateau is a model representation of

182 flora that is associated with seasonal monsoonal rains in the Western Ghats, as well as the

183 flora of lateritic plateaus of the Western Ghats. All field experiments were carried out across

184 three consecutive years - 2017, 2018 and 2019, between 15th September and 5th November.

185 The first two years focused on the study of plant-pollinator interactions and the third year

186 involved the study of the effect of nutrient resources on the gender of the flower, and also

187 monitored possible yearly fluctuations in gender expression.

188 *Gender distribution in the population*

189 To determine the extent of sexual dimorphism in morphological characters of *M. simplex*,

190 flower sizes were compared. Pairs of male and hermaphrodite flowers were collected from 15

191 random inflorescences and then arranged and photographed on a laminated graph sheet. From

192 these images, petal length and width (widest diameter) were measured manually (Appendix

193 S1: Fig. S1).

194   In order to identify if the natural gender distribution within a plant and within an

195 inflorescence were male dominant or hermaphrodite dominant, a multi-year census was

196 conducted within the Kaas plateau. All census were carried out in the years 2018 and 2019 at

197 an interval of seven days, across 4 weeks (spanning over one and a half months) covering the

198 peak flowering season of *M. simplex*. Gender census were conducted between 12:30 hrs. to

199 16:00 hrs. (anthesis time of *M. simplex* flowers), on marked inflorescences (N = 30). Since a

200   single individual can bear 2-5 inflorescences, a pooled census was taken from all the

201   inflorescences of a plant as our initial observations found no difference among the

202   inflorescences within a plant. To identify if there is a positional-gender bias i.e. from basal to

203   the apical position within an inflorescence (see Stephenson 1981, Diggle 1995, 1997, Cuevas

204   and Polito 2004) the positions of each flower within an inflorescence were recorded along

205   with its gender details, over the census duration mentioned above. The positions within an

206   inflorescence were divided into three categories- apical, middle, and basal as shown in Fig.

207   1c.

208   To test the effect of nutrient resources on gender expression, a floral gender census

209   was conducted among two populations that differed in the availability of water and other

210   resources (presence or absence of a stream and a deep soil substrate), which we observed to

211   be primary limiting resources on an otherwise homogeneous plateau. The first population is

212   henceforth referred to as the plateau population - 'Plateau_pop', while the second population

213   is referred to as the stream population - 'Stream_pop'. Plateau_pop was selected as the

214   resource-poor population whereas Stream_pop was selected as the resource-rich population.

215   The two locations were separated by ~576 meters and other than the presence of a stream and

216   a deep soil substrate, can be viewed as a single population. The census was carried out in

217   October 2019 on randomly selected individuals within these two populations (N = 100 per

218   location).

219   *Pollination biology*

220   Pollen is the only pollinator reward present in the genus *Murdannia* (Faden 2000, Nandikar

221   and Gurav 2015). To identify any quantifiable difference in the pollen or paternal

222   contribution between the male and hermaphroditic flowers, the amount of pollen per anther in

223   virgin flowers of *M. simplex* was quantified using a hemocytometer (Bechar et al. 1997).

224   Pollinator visitations on *M. simplex* in the wild were quantified by observing

225   pollinators in 2 ft. x 2 ft. observation arenas (N = 49) in multiple intervals of 15 minutes

226   between 12:30 hrs. to 16:00 hrs. (anthesis time of *M. simplex*). Each arena consisted of 4-10

227   inflorescences having 15-30 flowers per area. Visitation by a pollinator was recorded when a

228   pollinator was observed to land on the flower and actively collect pollen. The following three

229   features were recorded and quantified: total number of flowers present, type of pollinator, and

230   number of pollinator visits in a 15-minute interval. Pollinator visitation rate was then

231   calculated as the total number of visits per flower per hour. At the end of the flowering

232   season, fruitset was quantified for *M. simplex* by measuring the number of fruits in randomly

233   selected inflorescences from the study population in 2017 (N = 26) and 2019 (N = 40).

234   *Gender-specific pollinator preference experiment*

235   Manipulated choice experiments were designed and conducted in the wild in an open

236   manipulated setup to test: a) presence of visitation bias by pollinators towards male flowers

237   (M) and/or hermaphrodite flowers (H), and b) presence of floral-density dependent bias by

238   pollinators. It was hypothesized that if the pollinator has an inherent gender preference,

239   irrespective of the gender density in a population, pollinators will show high visitation to the

240   same gender. Floral distributions within an inflorescence were defined to have binary

241   outcomes i.e., male abundant or hermaphrodite abundant, or equally male and

242   hermaphroditic. The experimental setup consisted of two pots that were kept one foot apart

243   with a total of 5-15 flowers in each pot and pollinator visits were quantified by eye between

244   12:30 hrs. to 16:00 hrs. Three gender-density treatments: H < M (T1), H = M (T2) and H > M

245   (T3) were tested. The density of flowers of the two genders in each experimental set up was

246   manually controlled and each treatment was repeated for a total of eight trials and a total

247   observation time of ~21.5 hours (Appendix S1: Fig. S2). Unmanipulated, wild set up

248   observations served as the control. The mean visitation rate per flower to male flowers and

249   hermaphrodite flowers were calculated for each pollinator type, by dividing the total number

250   of visits by a specific pollinator with the total number of flowers present in the experimental

251   set up. Pollinator visitation rate was calculated as the total number of visits per flower per

252   hour.

253   The overall visitation observed in all three different density treatments in the

254   manipulative choice experiment were compared to test the effect of change in density on the

255   visitation pattern of pollinators in this andromonoecious species. The visitation rates in the

256   control treatments (natural visitations) were calculated and compared with the visitation rates

257   obtained in our choice experiment (H < M, H = M and H > M). The mean visitation rates

258   across all three treatments were also compared with the total number of flowers present in the

259   trial to identify any effect of floral display size.

260   *Statistical analysis*

261   All analyses were performed in R (R Core Team 2019) and raw data was tested for normality

262   before proceeding with statistical analysis using Shapiro-Wilk's method. When Gaussian

263   type distribution could not be assumed, non-parametric Kruskal-Wallis rank-sum tests were

264   run using the dplyr package (from tidyverse package; Wikcham et al. 2019). The floral

265   diameters and pollen densities per flower between male and hermaphrodite flowers were

266   compared using unpaired two-sample t-test. The variation in proportion of male and

267   hermaphrodite flowers was quantified using Kruskal-Wallis test, and post hoc Dunn's test

268   (Dinno 2017) was used to identify the significant differences in proportion of genders

269   between the four weeks. Simple linear regression was used to check the effect of mean

270   number of male flower, hermaphrodite flowers and total flowers on the fruitset respectively,

271   and the effect of total floral display size on the mean visitation rate across the three

272   treatments in the choice experiments. The alpha was set at 0.5 ($P<0.025$) for all statistical

273   analysis. All graphical plots were produced using the ggplot2 package in R (from tidyverse

274   package; Wikcham et al. 2019).

275   **Results**

276   *Gender distribution in the population*

277   Due to the small size and fragile nature of the flower, floral size was measured as the widths

278   of the flowers. The floral morphometry between the size of male and hermaphrodite flowers

279   showed no significant difference (t = 0.11072, df = 28, *p*-value = 0.9126; Appendix S1: Fig.

280   S1). Throughout the flowering season, male flowers were present in higher proportions than

281   the hermaphrodite flowers in all the study plots by at least 70-80% (Kruskal-Wallis chi-

282   squared = 38.707, df = 7, *p*-value = 2.223^{e-06}; Fig. 2a; Appendix S1: Table S1). A slight

283   decrease in the proportion of male flowers was observed only in week 4 (Mean±SE: Week 1

284   = 1.33±0.31, Week 2 = 1.6±0.29, Week 3 = 1.73±0.35, Week 4 = 0.87±0.22; Fig. 2a;

285   Appendix S1: Table S1). In contrast, the number of hermaphrodite flowers remained similar

286   across all four weeks of observation (Mean±SE: Week 1 = 0.60±0.21 , Week 2 = 0.33±0.12 ,

287   Week 3 = 0.50±0.27 , Week 4 = 0.40±0.22 ; Fig. 2a; Appendix S1: Table S1) and only plants

288   that were closer to the water source i.e. stream population, had a significantly higher number

289   of hermaphrodite flowers (Mean±SE: Plateau_pop H = 0.43±0.07 vs. Stream_pop H =

290   1.75±0.14; *p*-values = 0.0000; Fig. 3; Appendix S1: Table S2). Overall, the mean number of

291   total flowers remained consistent for the first two weeks (Mean±SE: Week 1 = 1.93±0.39,

292   Week 2 = 1.93±0.37 flowers; Fig. 2a inset), increased in week 3 (2.23±0.41 flowers) and then

293   reduced in week 4 (1.26±0.28 flowers; Kruskal-Wallis chi-squared = 3.2847, df = 3, *p*-value

294   = 0.3498; Appendix S1: Table S1).

295   Within an inflorescence, male and hermaphrodite flowers were present in all the three

296   positions i.e. apical, middle and basal positions (Fig. 1c), and the mean number of male

297   flowers varied across all these three positions during our four-week observation period. The

298   number of male flowers were relatively consistent in the basal position throughout the plants'

299   flowering season, than in the middle position or apical position (Fig. 2 b - e; Appendix S1:

300   Table S3, S4). We documented the highest number of male flowers in the middle position of

301   the inflorescence, whereas the number of hermaphrodite flowers was consistent at all

302   positions over the flowering season (Fig. 2 b - e; Appendix S1: Table S3, S4).

303   The total number of flowers was significantly higher in the stream population than in

304   the plateau population (Mean±SE: Plateau_pop = 2.78±0.12 vs. Stream_pop = 3.32±0.17; t =

305   -2.5646, df = 176.65, p-value = 0.0116; Fig. 3 inset) and the stream population also showed

306   similar proportions of male and hermaphrodite flowers (Mean±SE: Stream_pop H =

307   1.75±0.14 vs. Stream_pop M = 1.57±0.14; p-value = 0.1614; Fig. 3). Proportion of male

308   flowers was significantly greater in the plateau population over the stream population

309   (Mean±SE: Plateau_pop M = 2.35±0.12 vs. Stream_pop M = 1.57±0.14; p-value = 0.0000;

310   Fig. 3; Appendix S1: Table S2).

311   The mean fruit set per plant showed no effect with the total number of male,

312   hermaphrodite, or total number of flowers present per plant, respectively in the plateau_pop

313   (hermaphrodite: r^2 = -0.0457, df = 18, p-value = 0.6852; Fig. 4a; male: r^2 = -0.04999, df =

314   18, p-value = 0.761; Fig. 4b; total flowers: r^2 = -0.04254, df = 18, p-value = 0.6412; Fig. 4c),

315   and in the stream_pop (hermaphrodite: r^2 = -0.0.05543, df = 18, p-value = 0.9637; Fig. 4d;

316   male: r^2 = 0.1125 , df = 18, p-value = 0.08134; Fig. 4e; total flowers: r^2 = 0.04828, df = 18,

317   p-value = 0.1781; Fig. 4f), respectively.

318   *Pollination biology*

319   Flowers of both the genders opened between 12:30 hrs. to 16:00 hrs., and the mean pollen

320   count for both the genders ranged from 5000-10000 pollen grains per anther and no

321   significant difference in the pollen quantity between the two genders was observed (t = -

322   0.67913, df = 60, p-value = 0.4997; Azad 2018, Albal 2019, Nawge 2019). The major

323   pollinators to *M. simplex*, in the order of visitation rates from highest to lowest were *Apis*

324   *dorsata* (Mean number of visits per flower per hour±SE = 1.01±0.18), *Apis cerana*

325   (0.86±0.10)*, Apis florea* (0.76±0.22), and *Amegilla spp. (Zonamegilla*, 0.3±0.09)

326   respectively. *Amegilla spp.* showed significantly lower visitation than all the other pollinators

327   (Kruskal-Wallis chi-squared = 6.5702, df = 3, *p*-value = 0.09; *p*-values: *Amegilla spp.* vs *A.*

328   *cerana* = 0.0074, *Amegilla spp.* vs *A. dorsata* = 0.0058, *Amegilla spp.* vs *A. florea* = 0.0469),

329   whereas the visitation rates among *A. cerana, A. florea* and *A. dorsata* were not significantly

330   different (*p*-values: *A. cerana* vs *A. dorsata* = 0.3822, *A. cerana* vs *A. florea* = 0.4278, *A.*

331   *dorsata* vs *A. florea* = 0.3748; Azad 2018). Other pollinators observed were *Paragus spp.*

332   (Hoverfly), *Lasioglossum spp.*, *Pseudapis spp.* and two more species that we could not

333   identify, henceforth referred to as UnID sp. 3 and UnID sp. 4 (Fig. 5). Mean pollinator

334   visitation rates were observed to be high at the beginning of the anthesis (1.74-1.94 flowers

335   from 13:00 hrs. to 15:00 hrs.) and it decreased with time (0.94-1.32 flowers from 15:00 hrs.

336   till anthesis end; Azad 2018).

337   *Gender specific pollinator preference experiment*

338   In all the experimental setups in the manipulated choice experiment, pollinator visitations

339   were highest to the most abundant gender (Kruskal-Wallis chi-squared = 65.3103, df = 7, *p*-

340   value = 1.303^{e-11}; Fig. 6; Appendix S1: Table S5). Pollinator visitation rates were

341   significantly different between hermaphrodite flowers in the unmanipulated, wild set up

342   (control) and hermaphrodite flowers in all the experimental setups (Fig. 6; Appendix S1:

343   Table S5). However, the visitation rates to the male flowers in the control were not different

344   from those to male flowers in all the experimental setup (Fig. 6; Appendix S1: Table S5).

345   Display size (total number of flowers per trial) had no effect on the visitation rates in any of

346   the manipulated treatments (r^2 = -0.03765, df = 21, *p*-value = 0.6309; Appendix S1: Fig. S3).

347   Quantification of pollinator-wise visitation rates in the manipulated experiments show

348   that visitations by *Apis cerana, Amegilla spp.* and UnID sp. 4 were higher to male flowers in

349   treatment 1 where male flowers were more abundant than hermaphrodite flowers (Mean

350   visitation rate per flower per hr±SE:- H < M: *A. cerana* = 3.29±1.11, *Amegilla spp.* =

351   1.81±0.91, UnID sp. 4 = 2.49±1.89 flowers; Kruskal-Wallis chi-squared = 13.8517, df = 9, *p*-

352   value = 0.1277; Fig. 7a; Appendix S1: Table S6). In treatment 3, *Apis cerana*, *Amegilla spp.*,

353   and UnID sp. 4 showed high visitation to hermaphrodite flowers (H > M: *A. cerana* =

354   2.76±0.74, *Amegilla spp.* = 0.89±0.61, UnID sp. 4 = 0.66±0.56 flowers; Kruskal-Wallis chi-

355   squared = 9.2848, df = 5, *p*-value = 0.09823; Fig. 7c; Appendix S1: Table S6). We observed

356   visitation by hoverflies only in treatment 2 (H = M: Visitation rate per hermaphrodite flower

357   = 0.56, visitation rate per male flower = 0.75; Kruskal-Wallis chi-squared = 15.605, df = 11,

358   *p*-value = 0.1565; Fig. 7b; Appendix S1: Table S6).

359   **Discussion**

360   *Gender distribution in the population*

361   The optimal allocation hypothesis proposes that between a costly and a cheaper gender, in a

362   resource-limited environment, the gender which requires the least resources will be preferred.

363   From a plant's perspective, the male flowers are considered a cheaper gender to produce

364   because there is no investment towards fruit development, unlike the hermaphrodite flowers

365   which produces both viable pollen and ovules (Bertin 1982a, Anderson and Symon 1989,

366   Kaul et al. 2002, Narbona et al. 2002, Obeso 2002, Verdú et al. 2007, Zhang and Tan 2009).

367   Here, we show that the *M. simplex* population on Kaas plateau has a higher density of male

368   flowers and the only individuals that showed a slight reduction in the number of male flowers

369   and an increase in hermaphrodite flowers were the ones present along the stream. We

370   consider the stream population of *M. simplex* to have better resources available to them than

371   the plateau population because of the presence of water (a limited resource otherwise on the

372   plateau) and possible higher nutrient availability due to dissolved/associated nutrients in the

373   water and deeper soil substrate available for the plants (Watve 2013, Ramey and Richardson

374   2017, Thorpe et al. 2018). This suggests that although the general tendency of *M. simplex* is

375   to produce more male flowers over hermaphrodites, water and water-dissolved minerals may

376   be major limiting resources and in the absence of resource constraints the plants can produce

377   not only more flowers but also higher number of hermaphrodite flowers.

378   *Gender shift with time*

379   Resources available and utilized by plants often fluctuate with time and age of the plant

380   (Stephenson 1982, Solomon 1985). We observed an initial increase in the number of flowers

381   followed by a decreasing trend in the total number of flowers being produced per plant

382   throughout its flowering period (Fig. 2a inset). Within this trend, male flowers were always

383   produced in higher proportions than the hermaphrodite flowers and interestingly, in the end

384   of the flowering season (i.e. fourth week) we observed a reduction in the number of male

385   flowers instead of the number of hermaphrodite flowers (Fig. 2a). Thus, although resource

386   constraints do affect the production of total flowers as observed in the stream vs. plateau

387   population, resource limitation due to the flowering stage of the plant only affected the

388   number of male flowers in *M. simplex*. It has been shown that under declining resource levels

389   plants may opt to increase their fitness by producing the relatively inexpensive male flowers

390   over the expensive female flowers (May and Eugene Spears Jr 1988). Further, it has been

391   reported in *C. benghalensis* that a plant will opt to drop a flower rather than dropping a fruit,

392   under resource limiting conditions, because the nutrient resources that would get committed

393   to fruits that is aborted would be saved halfway (Kaul and Koul 2008). We propose that a

394   similar strategy may be operational in *M. simplex* wherein under availability of resources *M.*

395   *simplex* may be producing excess of male flowers which are the first ones to be sacrificed

396   when resource limitation starts to be an important factor as the phenology of the plant

397 progresses (Fig. 2a). The lack of change in the number of hermaphrodite flowers in different

398 stages of plant's flowering phenology also suggests that the hermaphrodite flowers may

399 already be produced at an optimal amount, resulting in the maintenance of their number

400 despite declining resources at the later stages (Fig. 2a).

401 Gender distribution of flowers on an inflorescence has been shown to be dependent on

402 resource allocation (Diggle 1995, 1997, Miller and Diggle 2003), and it has been observed

403 that the differential positioning of the genders within an inflorescence can affect outcrossing

404 rates (Orth and Waddington 1997, Lin and Forrest 2019). Studies in *Solanum* (Diggle 1994,

405 1995, 1997, Miller and Diggle 2003), *Caesalpinia* (Lewis and Gibbs 1999), *F. virginiana*

406 (Ashman and Hitchens 2000) and in other Commelinaceae genera (Faden 1991, Kaul et al.

407 2002, Kaul and Koul 2008) have shown that higher accessibility to nutrient resources at the

408 base of an inflorescence (than the distal end) can result in the presence of higher resource-

409 demanding hermaphrodite flowers in the basal position while males are relegated to the

410 middle and apical positions of an inflorescence. We found a similar pattern in *M. simplex,*

411 where a greater number of male flowers were produced at the middle and the apical positions

412 on an inflorescence. However, unlike other plants where hermaphrodites exclusively occupy

413 the basal positions in an inflorescence, in *M. simplex* hermaphrodites were also present in the

414 apical position. In fact, the distribution of hermaphrodite flowers was not significantly

415 different across all the three positions, over the course of the plant's flowering season. This

416 implies that any manipulation in display size is carried out by increasing or decreasing the

417 male flowers thus regulating the floral display at a much lesser cost (as proposed in *Solanum*

418 by Anderson and Symon 1989).

419 *Pollination biology*

420 For inflorescence size to affect reproductive success, the number of flowers visited per

421 inflorescence by pollinators must increase with display size because pollinators have been

422   shown to display higher preference to larger inflorescences (Stephenson 1981, Harder and

423   Barrett 1995, Ohashi and Yahara 2001). Hence the disproportionate gender distribution

424   within the inflorescence of an andromonoecious species may be a result of indirect pollinator

425   selection through inflorescence size: that is larger inflorescences are better at attracting

426   pollinators but under nutrient resource limitation, this can be achieved only by producing

427   more male flowers, which are cheaper to produce (Sandring and Ågren 2009). Thus our

428   results support the pollinator attractor hypothesis as proposed by Zhang and Tan (2009) and

429   sex allocation theory as proposed by Elle and Meagher (2000) where they predicted that male

430   flowers can increase the floral display and their attractiveness to pollinators at a lower cost

431   through male function than female function and thereby increase the stigmatic load of the

432   hermaphrodite flowers, which potentially increases female reproductive success at lower

433   resource investment.

434   The dominant pollinator assemblage of *M. simplex* consisted mainly of different

435   species of bees from the two genera of bees common in the Indian tropics - *Apis* and

436   *Amegilla*. All pollinators that visit *M. simplex* are generalists and are known to be common

437   pollinators in grassland habitats (Corlett 2011, Dhargalkar 2019, Nawge 2019). There are at

438   least 68 plants co-flowering with *M. simplex* on the Kaas plateau (Dhargalkar 2019) and

439   parallel studies on the pollination of plants in the Kaas community showed that both *Apis*

440   *cerana* and *Apis florea* switch to *M. simplex* from *M. lanuginosa* (anthesis time 10.30 hrs.-

441   13.00 hrs.) and various *Impatiens* species as soon as the flowers of *M. simplex* open i.e. at

442   12:30 hrs. up to 16:00 hrs. (Albal 2019). This suggests that although the pollinators are

443   generalists, these pollinators may be displaying temporal specialization towards *M. simplex*.

444   Future studies will focus on temporal specialization of the bee communities on Kaas plateau,

445   which will provide insight on pollinator preference towards *M. simplex* in a multi-species

446   environment.

447   *Gender-specific pollinator preference*

448   Several studies have shown that pollinators avoid functionally female flowers because of

449   decreased pollen or absence of pollen (Bierzychudek 1987, Eckhart 1991), floral size

450   (Ashman et al. 2000), floral scents (Ashman et al. 2005) and pollen-pistil interference

451   (Solomon 1985, Elle and Meagher 2000). However, in *M. simplex* both male and

452   hermaphrodite flowers showed comparable total pollen production. This has been shown in

453   other andromonoecious systems as well such as in *Solanum carolinense* (Solomon 1986),

454   *Olea europaea* (Cuevas and Polito 2004) and *Dichorisandra spp.* (Sigrist and Sazima 2015),

455   where the male flowers play a crucial role in the reproductive success of the plant by

456   increasing the total number of pollen grains available per plant. As previously discussed, we

457   observed that nutrient resources affected the number of male flowers and not the number of

458   hermaphrodite flowers, and the rate of fruit sets were observed to be similar between the

459   plateau and the stream populations (Mean±SE: Plateau_pop = 3.22±0.23 vs. Stream_pop =

460   3.55±0.21). This suggests that reproductive success is limited by the availability of

461   hermaphrodite flowers and not pollinator visitations. The lack of preference for either of the

462   gender by the pollinators on Kaas plateau is further supported by our observations from the

463   manipulative experiment where all pollinators except *A. cerana* displayed lack of preference

464   towards either of the floral genders. Further, this also fails to support the hypothesis that

465   male-dominant inflorescences of *M. simplex* may be under pollinator-mediated selection due

466   to direct pollinator preference for any gender (see Solomon 1985, Eckhart 1991, Elle and

467   Meagher 2000). However, since pollinator visitations were observed to be density dependent

468   (i.e. floral density, compare treatment 3 with treatment 1, Fig. 7c) it is possible that

469   pollinator-mediated selection is acting on male flowers through selection of larger

470   inflorescence sizes.

471   Our understanding of pollinator services by bees primarily come from *Bombus*

472   species (Chittka et al. 1997, 1999) and *Apis mellifera* (Martin 2004, Howard et al. 2019) and

473   studies on other bees from India are very rare (see Somanathan and Borges 2001). At least

474   five species of bees visited *M. simplex* and in the choice experiment, we observed inter-

475   species behavioral differences among bees both in their floral preferences and visitation

476   patterns, respectively. Almost all bees displayed higher visitation to any gender which was

477   present in greater density, suggesting that pollinator visitations are predominantly gender-

478   nonspecific and density-dependent. A slight preference for gender was only observed in *A.*

479   *cerana* and *Amegilla* in treatment 2 (H = M) where male flowers were preferred over

480   hermaphrodite flowers (Fig. 7b). Bees are known for both their short-term and long-term

481   memory retention and they can show learning through foraging experience (Cresswell and

482   Galen 1991, Schiestl and Johnson 2013) and we propose that the higher visitation to male

483   flowers in treatment 2 (Fig. 7b) may be a result of short-term memories and habituation in

484   bees since in natural conditions bees may encounter more male flowers than hermaphrodites.

485   We conclude that in *M. simplex* environmentally driven resource constraints play a

486   bigger role than pollinator preference towards a specific gender in determining gender

487   expression. However, we cannot dismiss an indirect role of pollinator-driven constraints

488   which may occur through selection on the display size as proposed in the pollinator attractor

489   hypothesis (Zhang and Tan 2009). In a resource-limited environment such as the one

490   observed at Kaas plateau, *M. simplex* can thus increase its display size by producing the less

491   expensive male flowers over hermaphrodite flowers.

492   Energy constraints will have a direct cost on floral production if genders require

493   differential resource allocation. As a result, it can be expected that the gender of the flowers

494   will be selected accordingly. Using the *M. simplex* system, we show that the gender

495   expression is directly influenced by the availability of nutrient resources in the plants'

496   environment and indirectly affected by the pollinators' preference for larger display sizes of

497   the inflorescence. Thus, a combination of both environmental factors as well as ecological

498   factors may be responsible for the evolution and maintenance of andromonoecy in mass-

499   flowering plants such as *M. simplex* in the tropics.

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Author contributions

511   VG conceived the idea, AA, AG, and VG designed the experiments. AA and AG conducted

512   the fieldwork with help from SS. AA performed the statistical analyses with inputs from SS

513   and VG. AA and VG wrote the manuscript and AG and SS helped with edits. All authors

514   read and approved the final draft of the manuscript.

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721 **Figure legends**

722 **Fig. 1** Habitat and floral features of *Murdannia simplex* (a) habitat on Kaas Plateau,

723 Maharashtra during flowering season (Inset: Location in India), (b) *M. simplex* habit, (c)

724 inflorescence image showing positions identified from base to the apex, (d) *M. simplex*

725 flower front view, (e) *M. simplex* male flower, (f) *M. simplex* hermaphrodite flower. Photo

726 courtesy: (a), (c), (d) - Asawari Albal, (b), (e), (f) - Saket Shrotri.

727 **Fig. 2** Variation in gender in *M. simplex* (a) by week from first flowering date (Kruskal-

728 Wallis chi-squared = 38.7067, df = 7, *p*-value = 0.00; P<0.025) (Inset: Week-wise proportion

729 of total number of flowers; Kruskal-Wallis chi-squared = 3.2847, df = 3, *p*-value = 0.3498, N

730 = 30; P<0.025) (See Appendix S1: Table S1) and (b) - (e) within-inflorescence from Week 1

731 - Week 4 respectively (Kruskal-Wallis chi-square test; df = 5, P<0.025). For (a) columns to

732 be compared within the week and within gender (Mean±SE). For (b) columns to be compared

733 within the gender and within positions, across all 4 weeks (Mean±SE). Significant *p*-values

734 depicted by different alphabets (Appendix S1: Table S3; P<0.025).

735 **Fig. 3** Natural gender distribution observed in *M. simplex* in 2019 between two populations

736 (Mean±SE) (Kruskal-Wallis chi-squared = 123.53, df = 3, *p*-value $<2.2^{e-16}$, N = 100 per

737 population; Appendix S1: Table S2; P<0.025) (Inset: Total number of flowers across the two

738 populations; t = -2.5646, df = 176.65, *p*-value = 0.0116). Male flowers are represented by

739 open squares, hermaphrodite flowers represented by filled squares and the gray squares

740 represent total (male + hermaphrodite) flowers. Columns to be compared within population

741 and within genders across the two populations only.

742 **Fig. 4** For plateau_pop in 2019, effect on mean fruitset by (a) mean number of male flowers

743 (r^2 = -0.04999, df = 18, *p*-value = 0.761, N = 20), (b) mean number of hermaphrodite

744 flowers (r^2 = -0.0457, df = 18, *p*-value = 0.6852, N = 20) and (c) mean number of flowers per

745 plant (r^2 = -0.04254, df = 18, *p*-value = 0.6412, N = 20). For stream_pop in 2019, effect on

746   mean fruitset by (f) mean number of male flowers ($r^2 = 0.1125$, df = 18, p-value = 0.08134,

747   N = 20), (e) mean number of hermaphrodite flowers ($r^2 = -0.05543$, df = 18, p-value =

748   0.9637, N = 20) and (f) mean number of flowers per plant ($r^2 = 0.04828$, df = 18, p-value =

749   0.1781, N = 20).

750   **Fig. 5** Pollinators of *M. simplex* from Kaas plateau: (a) and (b) *Amegilla spp. (Zonamegilla)*;

751   (c) *Apis florea*; (d) *Apis cerana*; (e) *Apis dorsata*; (f) *Paragus sp.* (Hoverfly); (g)

752   *Lasioglossum sp.*; (h) and (i) *Pseudapis sp.* Photo courtesy- (a) - (d), (f), (h), (i) : Asawari

753   Albal and (e), (g): Saket Shrotri.

754   **Fig. 6** Mean pollinator visitation rate per flower per hour (Mean±SE) observed in the

755   manipulative experiment where three treatments with varying numbers of hermaphrodite and

756   male flowers were used to test pollinator preference towards a gender; (Kruskal-Wallis chi-

757   squared = 63.4196, df = 7, p-value = 3.122^{e-11}, N = 8 trials for each treatment; P<0.025).

758   Male flowers are represented by open squares and hermaphrodite flowers are represented by

759   the filled squares. The first two columns represent the natural visitation rate observed per

760   flower per hour for both male and hermaphrodite, respectively. Different alphabets depict

761   significantly different values (Appendix S1: Table S5; P<0.025).

762   **Fig. 7** Pollinator-wise mean visitation rate per flower per hour in the manipulative experiment

763   on *M. simplex* (Mean±SE) (a) Treatment 1- H < M (Kruskal-Wallis chi-squared = 13.8517, df

764   = 9, p-value = 0.1277; P<0.025; Appendix S1: Table S6), (b) Treatment 2- H = M (Kruskal-

765   Wallis chi-squared = 15.605, df = 11, p-value = 0.1565; P<0.025; Appendix S1: Table S6),

766   (c) Treatment 3- H > M (Kruskal-Wallis chi-squared = 9.2848, df = 5, p-value = 0.09823;

767   P<0.025; Appendix S1: Table S6). Male flowers are represented by open squares and

768   hermaphrodite flowers are represented by filled squares. Different alphabets depict

769   significantly different values (Appendix S1: Table S6; P<0.025).
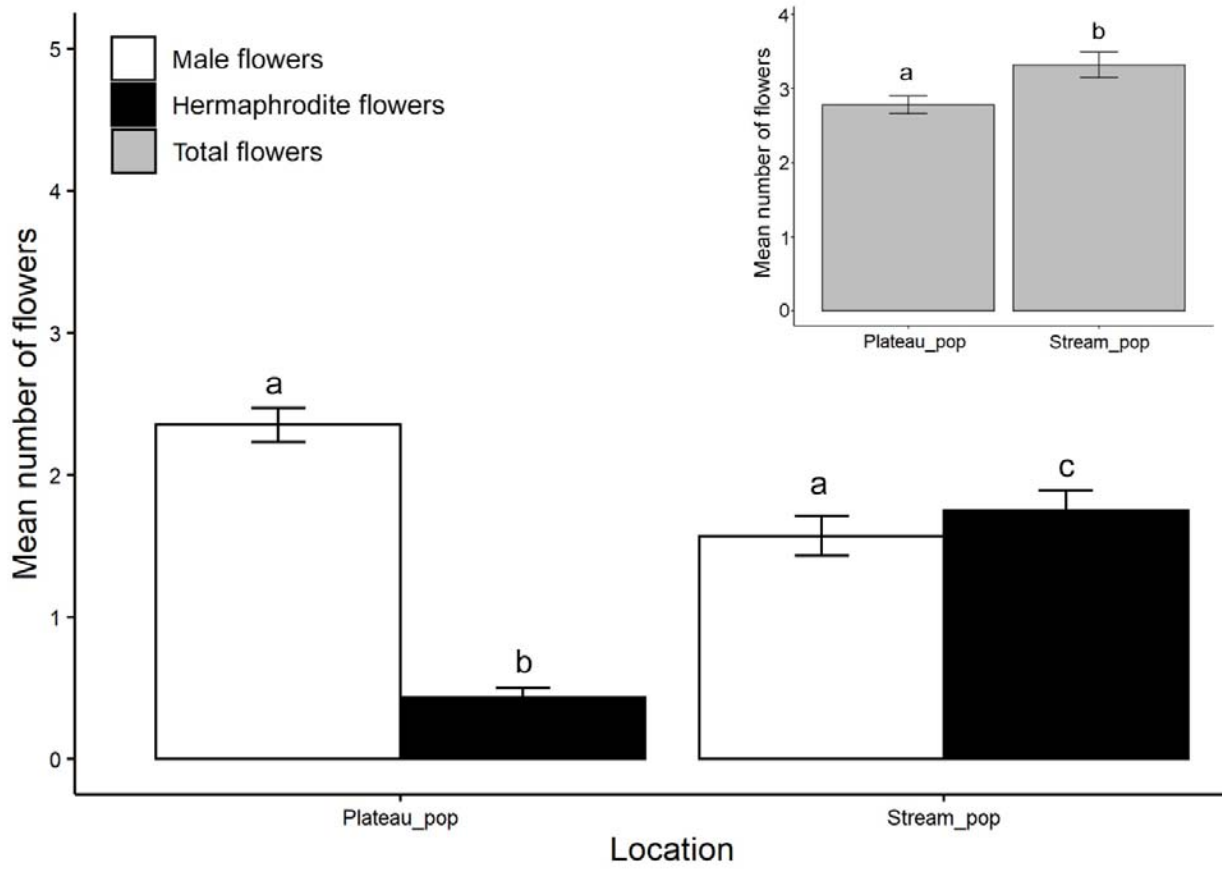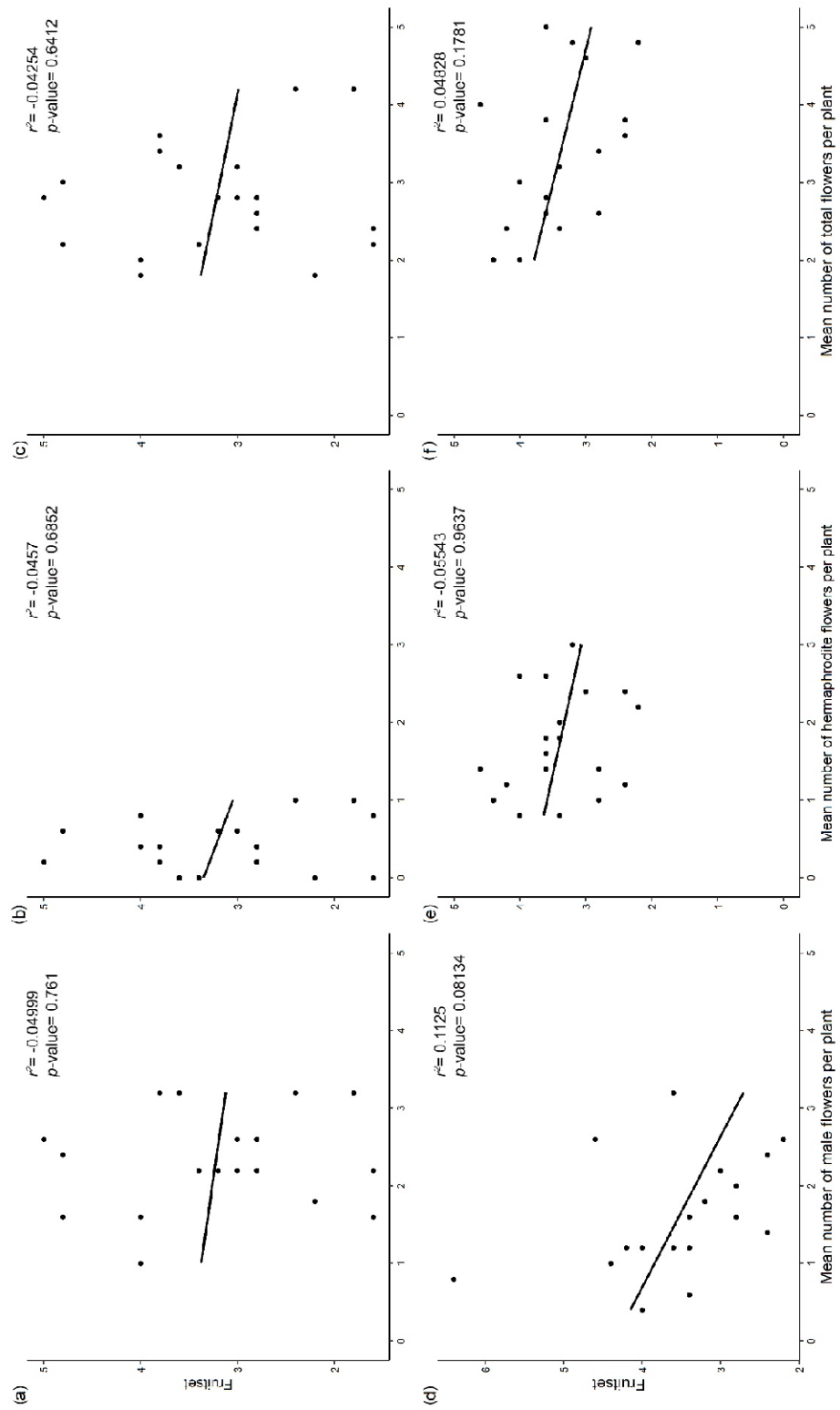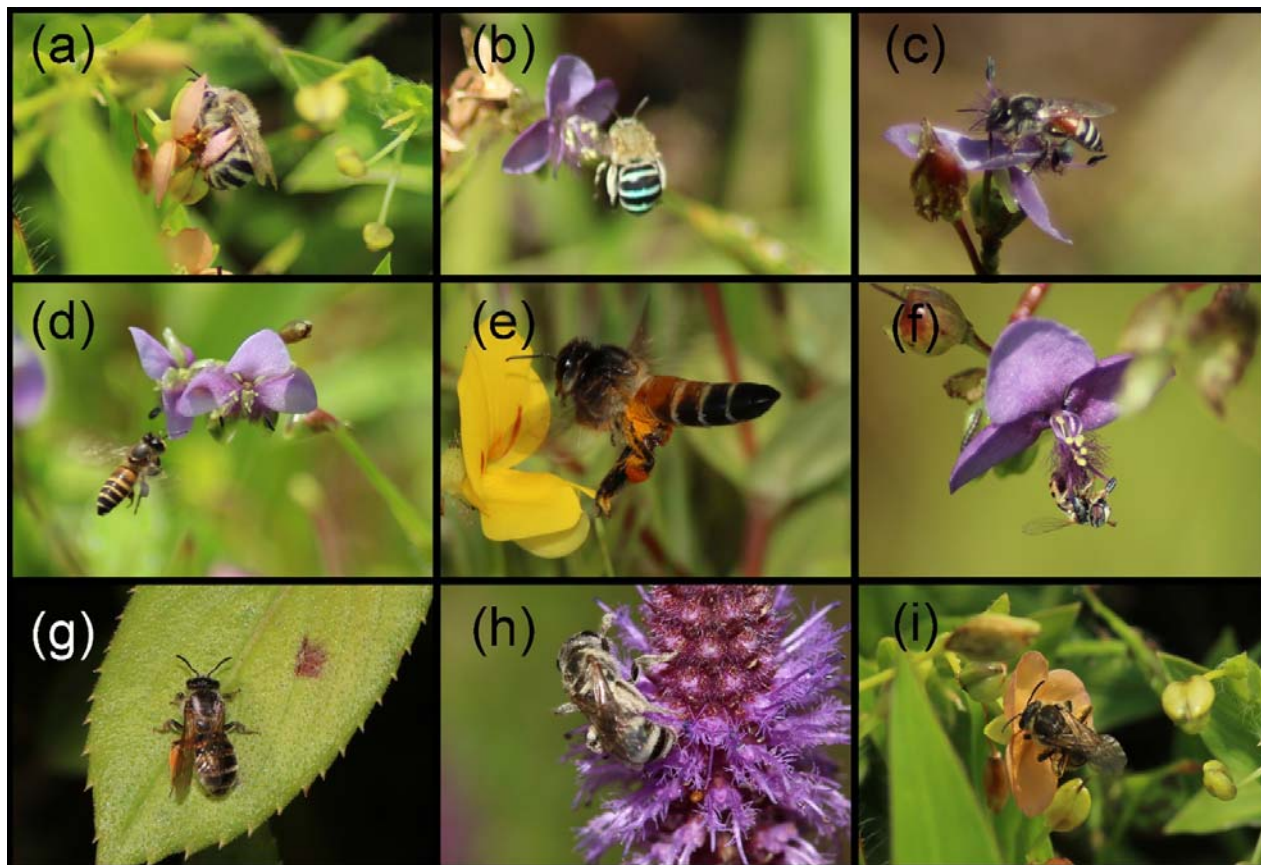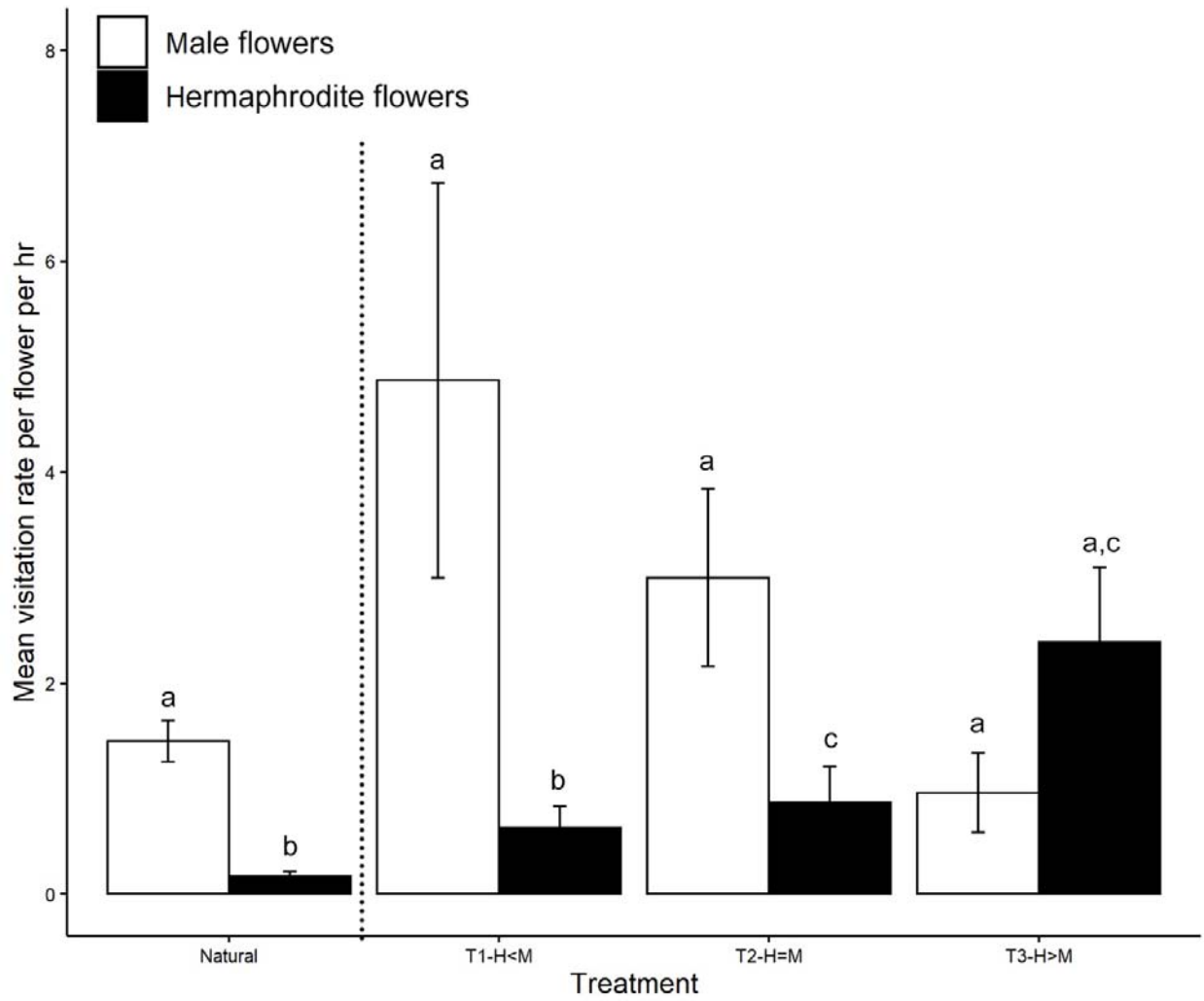
770 **Figures**
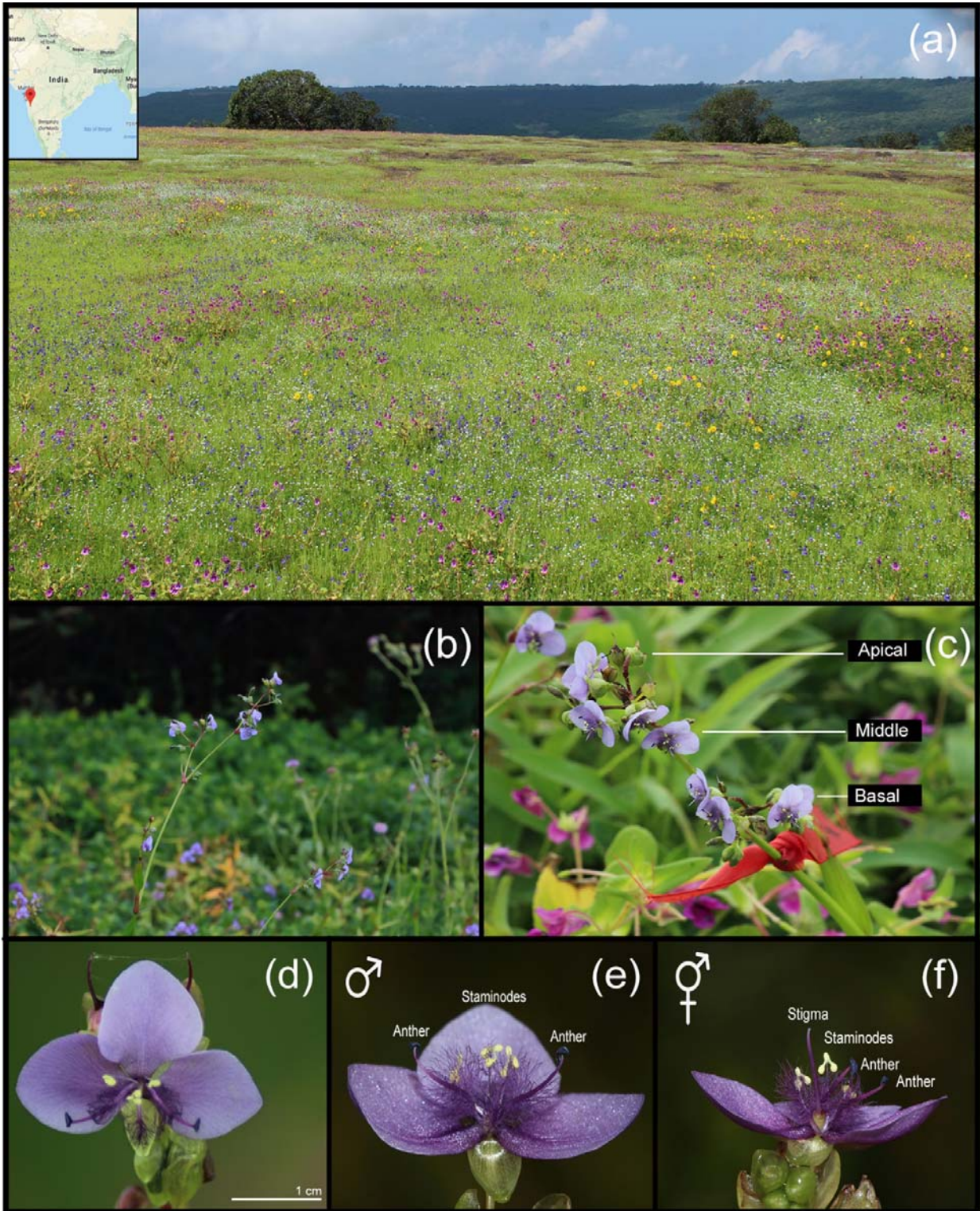
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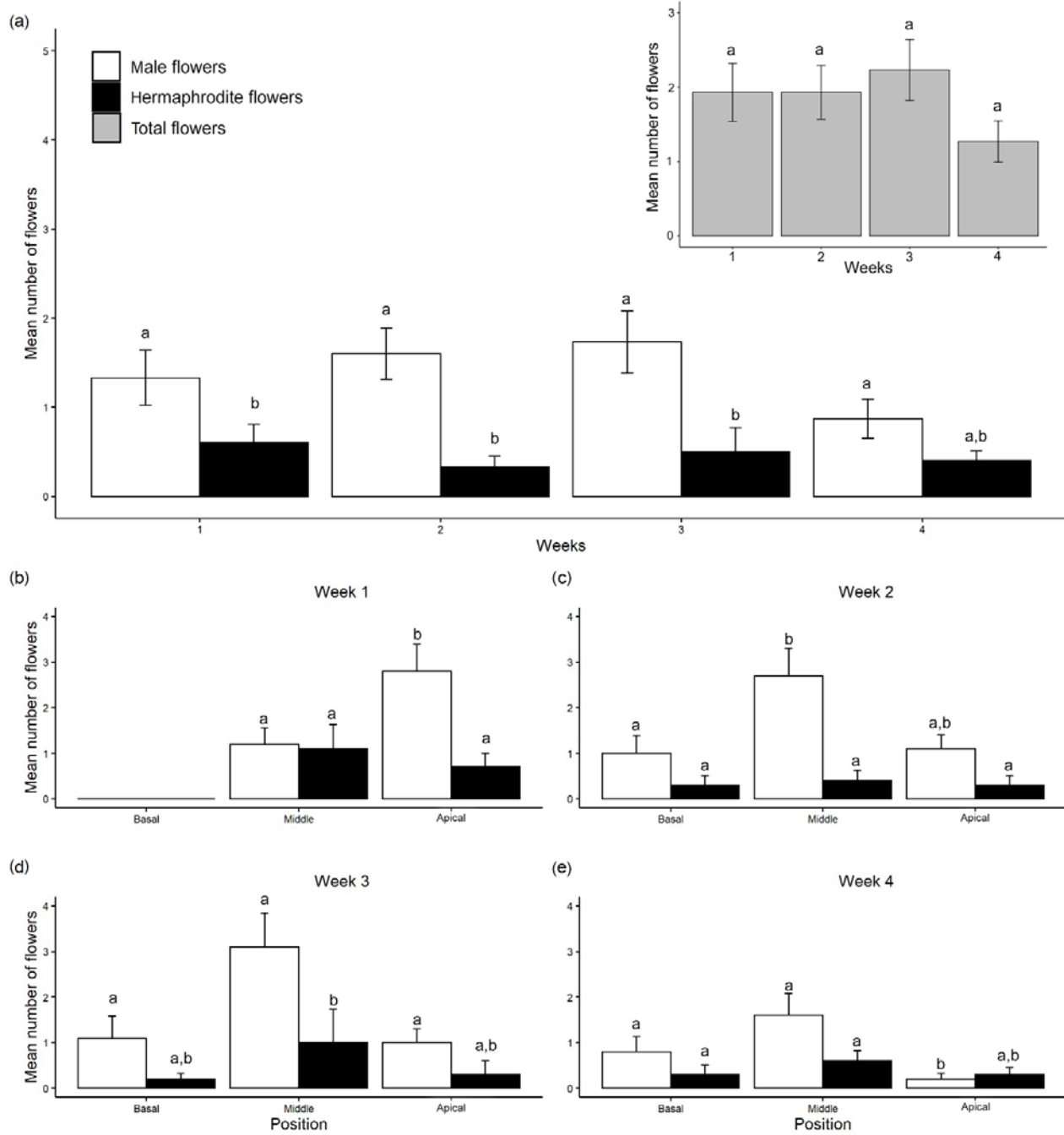
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