

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18

ORIGINAL ARTICLE

**ANDROMONOECY IN *SOLANUM LYCOCARPUM* A. ST. -HIL. (SOLANACEAE):
FLORAL ATTRIBUTES, VISITORS AND VARIATION IN SEXUAL EXPRESSION
OVER TIME**

Vanessa Gonzaga Marcelo¹, Vinícius Lourenço Garcia de Brito², Mario Vallejo-Marín³,
Hélder Consolaro⁴

¹Programa de Pós-Graduação em Biodiversidade Vegetal, Universidade Federal de
Goiás, Campus Samambaia, Instituto de Ciências Biológicas, 74690-900, Goiânia, Goiás,
Brazil.

²Instituto de Biologia, Universidade Federal de Uberlândia, 38400-902, Uberlândia, Minas
Gerais, Brazil.

³Department of Biological and Environmental Sciences, Faculty of Natural Sciences,
University of Stirling. FK9 4LA. Stirling, Scotland, United Kingdom.

⁴Departamento de Ciências Biológicas, Instituto de Biotecnologia, Universidade Federal de
Catalão, 75704-020, Catalão, Goiás, Brazil.

*Corresponding author: vanessamarcelo.bio@gmail.com

19 **ABSTRACT:** Sexual expression in andromonoecious species—those in which a single
20 individual can bear both staminate and hermaphroditic flowers—may vary among reproductive
21 events in the same plant, among individuals, and across populations. This variation influences,
22 in turn, the individual contribution of hermaphroditic plants via male and female fitness
23 functions (i.e., Lloyd’s *phenotypic gender*). However, temporal variation in sexual expression
24 in andromonoecious species and its relationship with seasonal changes in climatic conditions
25 remains poorly understood. Here we analyze floral attributes, visitors, and variation in sexual
26 expression in three populations of *Solanum lycocarpum* A. St. -Hil. Seasonality in the
27 production of floral types, the mating system, and floral visitors were also investigated.
28 Hermaphroditic flowers produced more pollen grains, but the pollen of staminate flowers had
29 higher viability. Only hermaphroditic flowers produced fruits, and ovules in staminate flowers
30 were sterile. *Solanum lycocarpum* is mainly pollinated by large bees with the ability to vibrate
31 flowers. Phenotypic gender varied throughout the year, and the seasonal production of
32 staminate flowers is associated with the local climate. We suggest that the higher and
33 seasonally-variable relative abundance of staminate flowers compared to the low and uniform
34 production of hermaphroditic flowers may be explained by (i) the very high energetic costs
35 incurred in producing large fruits which in turn make hermaphroditic flower production very
36 costly and, (ii) the potentially lower energy expenditure of the smaller staminate flowers with
37 reduced pistils and non-viable ovules that allow them to rapidly respond to climate variability.
38
39 **Keywords:** Buzz pollination, Cerrado, reproductive biology, sexual expression, sexual system.

40 INTRODUCTION

41 Andromonoecy is a sexual system in which the same individual produces hermaphroditic and
42 staminate (female-sterile) flowers (Cardoso et al., 2018; Diggle, 1994). Andromonoecy usually
43 evolves from hermaphroditic plants with hermaphroditic flowers by the loss of female
44 reproductive structures in a proportion of the flowers of the plant (Lewis, 1942). Andromonoecy
45 has evolved several times in the evolutionary history of angiosperms and occurs in about 4,000
46 species distributed in 33 phylogenetically unrelated families (Miller & Diggle, 2003;
47 Yampolsky & Yampolsky, 1922). The evolution of staminate flowers in andromonoecious
48 species is thought to allow individuals to distribute resources from pistil and ovule production
49 to other functions, including flower production and seed maturation in hermaphroditic flowers
50 (Anderson & Symon, 1989; Podolsky, 1992; Vallejo-Marín & Rausher, 2007a). Resource
51 allocation might be particularly important in species with large and fleshy fruits, in which
52 significant amounts of resources invested in floral and fruit development that ultimately fail to
53 reach full fruit maturation will be wasted (Lloyd, 1980a).

54 Previous studies have shown extensive variation in the proportion of staminate flowers
55 relative to the total flowers produced (sexual expression), at the species, population and
56 individual levels of andromonoecious plants (McDonnell, Wetreich, Cantley, Jobson, &
57 Martine, 2019; Miller & Diggle, 2003; 2007). For instance, within *Solanum* (Solanaceae), some
58 species produce relatively low proportions of staminate flowers and are considered weakly
59 andromonoecious [e.g. *Solanum hirtum* (<1% staminate flowers) (Diggle, 1993); *S. candidum*
60 (3%) and *S. ferox* (7%) (Miller & Diggle, 2003)], while others produce a higher proportion of
61 staminate flowers and are considered to be strongly andromonoecious [e.g. *S. pseudolulo* (39%)
62 and *S. quitoense* (60%) (Miller & Diggle, 2003)]. At the population level, the pattern of
63 variation is affected by the plastic developmental responses of individuals in a heterogeneous
64 environment (Diggle, 1991), including variation in water and soil nutrients availability (Albal,
65 Azad, Shrotri, & Gowda, 2020). At the individual level, sexual expression is also plastic and
66 can vary depending on the resource status, the size of the plant (Shwe, Wu, & Haung, 2020;
67 Whalen & Costish, 1986), the diameter and the number of fruits formed by individual (Diggle,
68 1991; May & Spears, 1988), herbivory damage (Krupnick & Weis, 1998; Reuther & Bockhoff,
69 2013), as well as pollination dynamics (Quesada-Aguilar, Kalisz, & Ashman, 2008).

70 Much less is known about the extent to which individual populations vary in the sexual
71 expression over time. Most studies with andromonoecious populations characterize only a
72 single sampling event, and thus do not allow evaluating temporal variation of individual

73 populations. We expect sexual expression to be sensitive to temporal variations in
74 environmental conditions (Calviño, Ashworth, & Moyetta, 2014; Diggle, 1993; Solomon,
75 1985) and thus might vary both within and among seasons. As plant phenological responses
76 such as flowering and fruiting are influenced by present and past local climatic variation
77 (Ehrlén, 2015; Johansson, Bolmgren, & Jonzén, 2013), it is expected that hermaphroditic and
78 staminate flower production vary along the year, especially in seasonal environments. This
79 variation would ultimately affect the phenotypic gender of the plants (i.e. the relative
80 contribution of individuals to the next generation as female and male parents; Lloyd, 1979).
81 Moreover, since hermaphroditic flowers are considered more costly to produce than staminate
82 flowers and staminate flowers are more susceptible to the climatic conditions over time
83 (NeSmith, Hoogenboom, & Groff 1994). Therefore, it is expected that staminate flower
84 production is more sensitive to variation in local climatic conditions than the production of
85 hermaphroditic flowers in andromonoecious plants. Such spatial and temporal dynamic of the
86 maleness expression of each individual is interpreted as a mechanism of cost savings in
87 investments and optimization of fruit development (Reuther & Bockhoff, 2013).

88 Although andromonoecy only occurs in about 2% of flowering plants (Richards, 1986;
89 Yampolsky & Yampolsky, 1922), it is better represented among species of the large (>1,500
90 species) genus *Solanum* (Solanaceae). Andromonoecy is widespread among the spiny *Solanum*
91 (Leptostemonum clade, *sensu* Särkinen, Bohs, Olmstead, & Knapp, 2013), which includes more
92 than 450 species distributed in the Americas, Asia, Africa and Australia (Bohs, 2005;
93 McDonnell et al., 2019; Whalen & Costich, 1986). *Solanum* is probably the group where
94 andromonoecy has been most extensively studied (e.g. Diggle, 1991), yet we still lack a clear
95 picture of the extent of variation in sexual expression of individual populations in the field.
96 Here we study the andromonoecious (Oliveira-Filho & Oliveira, 1988) species *Solanum*
97 *lycocarpum* A. St. -Hil. This species is abundant in the Cerrado (Brazilian savannah) across
98 very diversified environments, including anthropized habitats (Moura, Oliveira, & Chaves,
99 2010). Cerrado vegetation is subject to a strongly seasonal climatic regime with a very well-
100 defined rainy season (October to March) and a dry season (April to August). *Solanum*
101 *lycocarpum* (known as “fruta-do-lobo” or “lobeira”) has great ecological importance for the
102 Cerrado, as it offers pollen to a considerable number of floral visitors (Tavares, Alves-Junior,
103 Morais, Polatto, & Dutra, 2018) and fruits to many dispersing mammals, such as the maned
104 wolf (*Chrysocyon brachyurus*), crab-eating fox (*Cerdocyon thous*), tapir (*Tapirus terrestris*)
105 among others (Kuhlmann, 2018). As in most *Solanum*, the flowers of *S. lycocarpum* are
106 nectarless and have anthers that release pollen through small apical pores (poricidal anthers).

107 Plants with poricidal anthers are usually pollinated by bees capable of producing thoracic
108 vibrations to remove pollen, i.e., they are buzz-pollinated (Vallejo-Marín, 2019). However,
109 buzz-pollinated flowers are visited by a diverse range of floral visitors including buzzing and
110 non-buzzing bees the latter which may extract pollen through other means such as chewing
111 through the anther wall (Renner *Trigona*, Solis-Montero, Vergara, & Vallejo-Marín, 2015).
112 Characteristics of the floral visitor such as its size relative to the flower being visited, and
113 behavior (buzzing vs. non-buzzing) affect whether a floral visitor functions as a pollinator, a
114 pollen robber or somewhere in between (Solis-Montero et al., 2015; Solis-Montero & Vallejo-
115 Marín, 2017).

116 Our main goal in this study is to determine how seasonal changes affect the sexual
117 expression of *Solanum lycocarpum*. In addition, because of the limited information available
118 on the reproductive and pollination biology of natural populations of *S. lycocarpum*, we also
119 characterize the floral biology of both staminate and hermaphroditic flowers, as well as their
120 mating system and floral visitors. Our results provide insights into the temporal variation in
121 sexual expression of an important natural resource of the Brazilian Cerrado and contribute to a
122 better understanding of the plasticity in sexual expression in andromonoecious species.

123

124 MATERIALS AND METHODS

125 Study area

126 Three populations in central Brazil were selected for monitoring. The region where the three
127 areas are located has two defined seasons, a rainy summer (September to April) and a dry winter
128 (May to August) (Köppen, 1948, Alvares et al., 2013; Maruyama et al., 2019; Figure S1), and
129 average annual rainfall of 1,500 mm (Klink & Machado, 2005). The first population (UFG) is
130 in Pé-do-Morro Farm (Federal University of Catalão, Catalão, Goiás; 47°59'55.6" W and
131 18°06'51.9" S), at an elevation of 830 m a.s.l. and with average temperature always above 18
132 °C (Ferreira & Cardoso, 2013). The second population (CA) is located in a rural area of the
133 municipality of Campo Alegre de Goiás, Goiás, (47°62'48.37" W and 17°76'09.34" S) at 760
134 m a.s.l. and average annual temperature of 20 °C (Peixoto, 2015). The third population (OZN)
135 is located on Pontinhas Farm, located ten kilometers from the municipality of Orizona, Goiás
136 (48°23'30.9" W and 16°98'10.9" S), at 860 m a.s.l. and with an average temperature of 22 °C
137 (Ascheri, Moura, Ascheri, & Junior, 2009; Guimarães, 2008). As *S. lycocarpum* is very
138 common in these areas, samples previously collected from these areas and deposited in the UFG

139 herbarium (UFG 24361. Rizzo, J.A. et al., 13058 in 2005) and in Embrapa herbarium (CEN
140 105156. Rocha, G.P.E. 441 in 2015), were used as vouchers for this work.

141 **Floral biology**

142
143 Fifteen flowers were marked in each of five individuals of *S. lycocarpum* in the OZN population
144 in order to record the time of anthesis. The separation of the corolla lobes was used as criterion
145 for identification of floral opening. Withering, loss of brightness and color change of the whorls
146 were considered parameters of senescence.

147 Pollen quantity and viability were assessed for each floral type. To estimate pollen
148 quantity, 15 hermaphroditic and 15 staminate buds were collected from 15 individuals in each
149 population (one bud of each type per individual). The buds were fixed in 70% alcohol and the
150 number of pollen grains per anther was counted using a Neubauer chamber (Maêda, 1985).
151 Pollen counts per anther were multiplied by five to estimate the total number of pollen grains
152 per flower. Since anthers are similar in size, only one randomly-collected anther per flower was
153 analyzed. Pollen viability was estimated by acetocarmine staining (Radford, Dickison, Massey,
154 & Bell, 1974) on 100 pollen grains per slide. One anther from each of the 15 hermaphroditic
155 and 15 staminate buds was used in this case (one bud of each type from 15 individuals in each
156 one of the three populations). To identify the floral type of the buds, they were superficially
157 opened, in addition to the hermaphrodite buds that are usually in the first positions in the
158 inflorescence. Stained grains were considered viable and non-stained grains, non-viable. For
159 ovules number, one bud of each floral type was collected from ten individuals in each
160 population. Ovules present in each carpel were counted using a stereo microscope, and the result
161 was multiplied by two because ovaries are composed of two carpels.

162 Fifteen hermaphroditic and 15 staminate flowers were collected from each of 15
163 individuals in each population for the morphological characterization of floral types. The
164 collection of flowers was random and the flowers were in different positions in the
165 inflorescence. Samples were fixed in 70% alcohol for later measurements. In the laboratory,
166 the flowers were photographed, and digital images of the flowers were measured using *Image*
167 *J* version 1.51 (Rasband, 2016). We measured the length of the corolla as the distance from the
168 tip of the upper petal to the fusion of both basal petals and the width of the corolla as the distance
169 from the tip of one basal petal to the tip of the other basal petal (Solís-Montero & Vallejo-
170 Marín, 2017). Anther length and width (at the widest portion of the anther) were measured in
171 the two floral types. Style length (including stigma) was also measured.

172 **Mating system**

173 Fifteen hermaphroditic and 15 staminate flowers per treatment, of 15 different individuals, were
174 used for the study of the mating system, totaling 150 tested flowers in the OZN population.
175 These pre-anthesis flowers were bagged with mesh bags, when necessary, and subjected to the
176 following treatments: 1) Manual self-pollination: manual deposition of pollen from anthers on
177 the stigma of the same flower (both hermaphroditic and staminate flowers); 2) Cross-
178 pollination: flowers pollinated with pollen from flowers of different and distant individuals
179 (mixture of pollen of the two floral types); 3) Natural pollination (control): flowers which were
180 only marked and left available to pollinators; 4) Spontaneous self-pollination: buds which were
181 only bagged; and 5) Agamospermy: emasculated flowers. The results obtained for the mating
182 system were also used to confirm floral sexuality.

183 **Pollination biology**

184 Direct field observations were made in order to investigate the abundance and richness of
185 pollinators, in addition to the time and behavior of visitors. To assess the time of the visits, 24
186 hours of focal observations were made in the UFG population, divided into two 12-hour periods
187 (6:00 am to 6:00 pm) in non-consecutive days, with 30 minutes of observation dedicated to
188 each individual. In order to identify the most frequent visitors in the three populations,
189 additional observations were made in different months of the year, totaling 20 hours in each
190 population.

191 Floral visitors were collected using an entomological net, sacrificed in ethyl acetate,
192 frozen and fixed for identification. To assess the relationship between the size of the floral
193 visitor and the visiting behavior in floral types, the specimens were photographed beside a ruler,
194 which was used as a scale. Afterwards, the photographs were analyzed, and the insects had the
195 following parameters measured in the *Image J* software version 1.51 (Rasband, 2016): length
196 and width of the thorax, length and width of the abdomen, and total length.

197 **Temporal variation in sexuality**

198 Thirty individuals in each population were marked and monitored monthly from January to
199 December 2017, totaling 90 individuals in the three populations. In the flowering and fruiting
200 phenophases, the presence of open flowers and the presence and quantity of fruits were
201 evaluated. In addition to phenological monitoring, the number of hermaphroditic and staminate
202 flowers (floral types) was counted. Visual identification of floral types was easy because

203 hermaphroditic flowers have a well-developed pistil, while staminate flowers have a reduced
204 pistil. Afterwards, the number of each floral type and the estimated number of viable pollen
205 grains and functional ovules produced by each floral type was used to calculate the phenotypic
206 gender (G) of each plant by the following equation (Barrett & Harder, 2006, Lloyd, 1980b):

$$207 \quad G = \frac{d_i}{d_i + l_i \cdot \frac{\sum_i d_i}{\sum_i l_i}}$$

208 where d_i corresponds to the total number of functional ovules produced by the individual and l_i
209 corresponds to the total number of viable pollen grains. Since this equation weights the
210 phenotypic gender by the total amount of pollen and ovules produced by the population and the
211 number of flower types fluctuated along the studied year, we recalculated the phenotypic gender
212 of each plant in each month. We also calculated G considering the total number of viable pollen
213 grains and ovules produced by each plant during the year. G values can vary from 0 to 1, which
214 represents strictly male and female plants, respectively.

215

216 **Statistical analysis**

217 Morphometric data were analyzed using a two-way ANOVA to compare floral types and
218 populations. A generalized linear mixed model (GLMM) with Poisson distribution was used to
219 determine which factors influenced pollen availability, measured as the total number of pollen
220 grains in the flower buds. In the case of pollen viability, a GLMM with binomial distribution
221 was used instead, since pollen viability is measured as the presence or absence of stain in the
222 pollen grains. In both analyses, the floral type was considered the fixed factor and population
223 the random factor. A Chi-square test was performed to compare the number of visits between
224 floral types. We also used a Chi-square test to compare the number of fruits produced by
225 hermaphroditic flowers in Cross- and Natural pollination treatments. The function *glmer* was
226 used for running the GLMMs from the *lme4* package (Bates, Mächler, Bolker, & Walker, 2015).
227 Statistical significance was assessed using Type III sum of squares using the *Anova* function
228 from the *car* package (Fox & Weisberg, 2011).

229 To assess the flowering phenology of the floral types, seasonal variations were analyzed
230 using circular statistics (Morellato, Alberti, & Hudson, 2010; Zar, 2010). In this analysis, the
231 year was represented as a 360-degree circle and each month as a 30-degree arc. We considered

232 Jan=0°, Feb=30°, March=60° and so forth until Dec=330°. Afterwards, we used the Rayleigh
233 test, to examine whether the number of flowers produced are uniformly distributed around the
234 year (Morellato et al., 2010). The resulting r vector corresponds to the concentration around the
235 mean angle and can be taken as a measure of phenological seasonality, ranging from 0 (no
236 seasonality) to 1 (when all species are reproducing at the same time) (Morellato et al., 2000).

237 As hermaphroditic flower production was not seasonal (see below), we further analyzed
238 the influence of local climatic conditions only in the production of staminate flowers. To do
239 this, we used the mean number of staminate flowers produced in each month as response
240 variable and the climatic condition (dry or humid) of each month as the explanatory variable to
241 run a Watson-Wheeler test for homogeneity on two samples using the *circular* package. This
242 test is a non-parametric test to compare two samples with circular distribution. All analyses
243 were performed using R version 4.0.1 (R Development Core Team, 2020).

244

245 **RESULTS**

246 **Floral biology**

247 *Solanum lycocarpum* has flowers grouped in inflorescences, of the helical monocasial type, and
248 generally one to two flowers open per inflorescence per day. The flowers have a green calyx
249 with trichomes and divided into three to five sepals. Young flowers are blue and lighten into
250 violet hues over their lifespan. These flowers are pentamerous, actinomorphic and
251 gamopetalous and have a plicate corolla. The five stamens are bright yellow in anthesis,
252 grouped in a cone and have poricidal dehiscence (Figure 1). Pollen is the only resource offered.
253 The pistil has an ovoid greenish stigma that differentiates it from the style, becoming shiny
254 when receptive. The stamens are slightly curved, facing upwards and arranged on the lower
255 surface of the visitor's body during the visit. Most flowers of *S. lycocarpum* open from 6:00 to
256 9:00 h, others open throughout the day, but all flowers close at 18:00 h. The next morning, they
257 open again and stay open until 18:00 h. After that, the flowers change color and the petals
258 wither. The flowers have a longevity of approximately two days.

259 Hermaphroditic flowers have stamens and a long pistil (Figure 1a), while staminate
260 flowers also have stamens but a reduced pistil (pistillode) (Figure 1b). Moreover,
261 hermaphroditic flowers are usually located at the base of the inflorescence, being the first to
262 open. The length ($F = 27.74$, $df = 1$, $p < 0.001$) and width ($F = 16.73$, $df = 1$, $p < 0.001$) of the

263 corolla of hermaphroditic flowers were greater than those of staminate flowers (Table 1).
264 Anther length did not differ between floral types ($F = 0.26$, $df = 1$, $p = 0.61$) (Table 1), but
265 anther width was greater in hermaphroditic flowers ($F = 6.63$, $df = 1$, $p = 0.01$) (Table 1).
266 Staminate flowers have a reduced pistil, so that the style and stigma do not project beyond the
267 cone of anthers (Figure 1b); in turn, the style and stigma in hermaphroditic flowers are exerted
268 by about four millimeters above the anther cone (Figure 1a). Thus, the style is longer in
269 hermaphroditic than in staminate flowers ($F = 1684.76$, $df = 1$, $p < 0.001$). There was no
270 difference in floral attributes among populations (Table 1).

271 Hermaphroditic flowers produced more pollen grains than staminate flowers (Table 2).
272 Hermaphroditic flowers of *S. lycocarpum* produced $1.85 \times 10^6 \pm 0.87 \times 10^6$ (mean \pm sd) while
273 staminate flowers produced $1.74 \times 10^6 \pm 0.71 \times 10^6$ (mean \pm sd) pollen grains. Regarding pollen
274 viability, staminate flowers had more viable pollen (78 ± 22 % (mean \pm sd)) than hermaphroditic
275 flowers (56 ± 33 % (mean \pm sd)) (Table 2). Staminate flowers also had fewer and smaller ovules
276 (141 ± 32 (mean \pm sd)) than hermaphroditic flowers (395 ± 99 (mean \pm sd)) (Table 2). There
277 was also a difference in the amount and viability of pollen, and in the number of ovules
278 produced in populations and in floral types between populations (Table 2).

279 **Mating system**

280 Fruits were produced only through natural and cross-pollination in hermaphroditic flowers
281 (Table 3). There is no evidence that these treatments produce different number of fruits ($\chi^2 =$
282 0.22 ; $df = 1$; $p > 0.64$).

283 **Pollination biology**

284 All visits were made by bees, especially those with the ability to produce vibrations to remove
285 pollen (Figure 1c, d). In total, 269 visits were observed in 24 hours of observation. Floral
286 visitors included 16 species of bees in the families Apidae, Andrenidae and Halictidae. These
287 bees had varied sizes, from 7.37 to 29.86 mm (15.75 ± 6.29 mm; mean \pm sd) in total length
288 (head, thorax and abdomen) (Table S1). There was a higher frequency of visits between 12:00
289 pm and 03:00 pm h. Individuals from two species of bees that are unable to produce vibrations
290 to remove pollen from flowers were observed collecting pollen on flower petals (*Apis mellifera*
291 and *Trigona spinipes*).

292 The species of floral visitors were similar in the three populations, but the frequency of
293 visits was different (Table S1). In the UFG population, the bees responsible for most visits were

294 *Epicharis flava* and *E. analis* (27% of the total visits), followed by *Augochloropsis* sp. (22%),
295 *Bombus* spp. (*B. morio*, *B. brevivillus*, *B. pauloensis*) (17%), *Oxaea* cf. *flavescens* (16%), and
296 *Exomalopsis fulvofasciata* (6%). In the CA population, the bees that made the most visits were
297 *O. flavescens* (26%), *E. fulvofasciata* (22%), *Augochloropsis* sp. (21%) and *Ephicaris* sp. (4%).
298 In the OZN population, the bee *Centris scopipes* was responsible for 29% of the total visits,
299 and the other bees were *Xylocopa suspecta* (17%), *O. flavescens* (16%), in addition to *Ephicaris*
300 sp. and *E. fulvofasciata* with 10% of the visits each.

301 We did not detect differences among floral types in the number of visits they received.
302 In individuals with both types of flowers, it was observed that 24% of the staminate flowers
303 were visited (n = 126 flowers visited out of the 516 flowers present in the observed individuals),
304 while 20% of the hermaphroditic flowers were visited (n = 9 flowers visited out of the 45
305 flowers present in the observed individuals). There was no evidence that staminate flowers were
306 more frequently visited than hermaphroditic ones, as they were visited according to the
307 expectation for the number of flowers of each floral type in the population ($\chi^2 = 0.299$, df = 1,
308 p = 0.584).

309 **Temporal variation in sexuality**

310 Although some individuals produced staminate and hermaphroditic flowers throughout
311 the year, most individuals are functioning as pollen donors (male phenotypic gender) at any one
312 point in time across all three populations studied, no matter if the month was dry or humid
313 (Figure 2). A subset of individuals (17/30 in CA, 9/30 in OZN and 7/30 in UFG) remained
314 functionally male and did not produce any hermaphroditic flower (Figure 2). In individuals with
315 both sexual functions, there is continuous variation in female function, and there are no plants
316 producing only hermaphroditic flowers along the year (Figure 2).

317 Flowers and fruits were produced throughout the year (Figure 3, Figure S2).
318 Considering the three populations, there was a greater production of staminate flowers per
319 individual in the rainy season (r = 0.34, p < 0.01; Figure 3a), with a peak in February (mean
320 angle \pm angular sd = $44.85 \pm 1.15^\circ$) when individuals produced 13.9 ± 17.4 staminate flowers
321 (mean \pm sd). The production of hermaphroditic flowers per plant was uniform and did not have
322 a clear peak along the year (r = 0.20, p = 0.43; Figure 3b). The number of hermaphroditic
323 flowers produced per plant in each month was 0.18 ± 0.57 (mean \pm sd). Fruit set per plant was
324 also seasonal (r = 0.20, p < 0.01; Figure 3c), with a peak in July (mean angle \pm angular sd =
325 $184.40 \pm 1.26^\circ$) when plants bore 1.68 ± 3.82 fruits (mean \pm sd).

326 The mean number of staminate flowers per plant depends on the climatic condition
327 (W=40.795, df=2, $p < 0.01$; Figure 4). When the climate was humid, the mean number of
328 staminate flowers per plant was 1.6 times the number of flowers produced when the climate
329 was dry (Figure 4).

330

331 **DISCUSSION**

332 *Solanum lycocarpum* is one of the most representative plant of the Brazilian savannah. Our
333 study showed that at least 17 bee species in three populations forage on pollen of staminate and
334 hermaphroditic flowers. Despite morphological differences found between the two flower types
335 and the higher proportion of viable pollen grains in staminate flowers, bee visitors did not
336 discriminate between staminate and hermaphroditic flowers. Plants depends exclusively on the
337 bee pollination services to set fruits. The sexual expression in populations of *S. lycocarpum*
338 changes throughout the year affecting plant phenotypic gender and, despite needing more tests
339 for confirmation, it is tempting to state that these changes are associated with climatic
340 conditions experienced in the populations. One possible explanation to the higher and
341 seasonally-variable relative abundance of staminate flowers compared to the low and uniform
342 production of hermaphroditic flowers could be (i) the very high energetic costs incurred by
343 producing the large fruits that characterize this species which make increasing hermaphroditic
344 flower production very costly, as well as by (ii) the potentially lower energy expenditure of the
345 smaller staminate flowers with reduced pistils and non-viable ovules compared to
346 hermaphroditic flowers that allow individual plants to take advantage of resource variability
347 more effectively.

348 **Floral and pollination biology**

349 We found that in *S. lycocarpum*, hermaphroditic flowers are larger and produce about 7% more
350 pollen grains than staminate flowers. In contrast, the pollen of staminate flowers has a slightly
351 higher viability than the pollen of hermaphroditic flowers. The differences between pollen
352 viability and quantity in floral types in *S. lycocarpum*, make it difficult to determine if staminate
353 or hermaphroditic flowers may disproportionately contribute to fitness via male function in per
354 flower basis. Enhanced male function in staminate vs hermaphroditic flowers has been
355 proposed as an adaptive explanation for the evolution of andromonoecy (Elle & Meagher,
356 2000), although the evidence in support of this hypothesis is mixed even for the same species

357 (Quesada-Aguilar et al., 2008; Vallejo-Marín & Rausher, 2007b). The enhanced male function
358 and resource-reallocation hypotheses are non-mutually exclusive, and could both jointly
359 contribute to the selective advantages of producing female-sterile flowers in andromonoecious
360 species.

361 Self-incompatibility in *S. lycocarpum* highlights the importance of biotic vectors for
362 pollen transfer (Oliveira-Filho & Oliveira, 1988). We found no difference in the number of
363 visits between floral types, indicating that bee visitors do not make distinction between one
364 floral types. Similarly, a study with andromonoecious *S. carolinense* found no difference in
365 pollinator visitation or siring success between hermaphroditic flowers and experimentally
366 produced staminate flowers. *Solanum lycocarpum* is a buzz-pollinated species visited by bees
367 of different sizes (from 7 to 33 mm). Our study shows that pollinator behavior differs among
368 bee species. Bees of smaller body size (10 and 11 mm in length), such as *Augochloropsis* sp.
369 and *Exomalopsis fulvofasciata*, grabbed the anthers laterally and individually vibrated them,
370 with little or no contact with the stigma. This behavior was also observed in short-pistil
371 (staminate) flowers, but it occurred mainly in long-pistil (hermaphroditic) flowers. The
372 presence of the pistil above the cone prevents the bees from handling all the anthers at once
373 (sexual interference). Bees of larger body size, such as *Oxaea flavescens*, *Ephicaris* sp., *Centris*
374 *scopipes* and *Xylocopa suspecta* (17 to 30 mm in length), vibrated the anther cone, and in this
375 case, contact between the stigma and the visitor's abdomen occurred. In another study with *S.*
376 *lycocarpum*, the most efficient bees on the first visit in terms of fruit formation were those of
377 largest body size (Tavares, Alves-Junior, Morais, & Polatto, 2017). These findings indicate that
378 body size is an important factor for effective pollination in *S. lycocarpum*. In andromonoecious
379 *S. carolinense* large- and small-sized bees differ in their capacity to transfer and deposit pollen
380 in staminate vs. hermaphroditic flowers (Quesada-Aguilar et al. 2008). In *Cimicifuga simplex*
381 different pollinator environments affected the sex expression in different populations (Toji &
382 Itino, 2020). Therefore, the identity and size of pollinators of andromonoecious species might
383 be an important determinant of the reproduction of these species, and potentially affect the
384 selective pressure on the production of staminate vs. hermaphroditic flowers.

385

386 **Temporal variation in sexuality**

387 Andromonoecy is likely a mechanism of resource allocation in which staminate flowers, with
388 their reduced ovaries and lower energy investment in the production of smaller petals, save and
389 distribute energy to be redistributed to other functions in the plant, such as vegetative growth,

390 storage, and/or increased reproductive potential (Bertin, 1982; Elle, 1999; Emms, 1993; Miller
391 & Diggle, 2007; Primack & Lloyd, 1980; Solomon, 1986; Spalik, 1991). If resources are
392 unpredictable in previous stages, the ability to regulate the investment during the fruiting stage
393 must be advantageous, because fruit development represents a significant drain of the available
394 resources (Lloyd, 1980a). Thus, if non-production of viable pistils and ovules in staminate
395 flowers is related to the allocation of the available resources, species with large fruits should be
396 more strongly andromonoecious than those with small fruits, since more resources are needed
397 for fruit maturation (Miller & Diggle, 2003; Symon, 1979).

398 Phenotypic gender of individuals of *S. lycocarpum* varied over time and among
399 populations. It was found that within one year, there was a plastic response to environmental
400 variations in the studied populations, since the production of staminate flowers was higher in
401 the rainy season and lower in the dry season, while production of hermaphroditic flowers was
402 uniform. Although our study sampled a single year, the seasonality in flower production we
403 observed was clearly related to the local climatic conditions, demonstrating the influence of
404 local climatic variations in the production of floral types. Studies have shown that the
405 distribution of floral types may be sensitive to environmental conditions (Primack & Lloyd,
406 1980) and/or changes in the availability of resources for reproduction (Diggle, 1994; May &
407 Spears-Jr, 1988; Miller & Diggle, 2003). In the andromonoecious species *Cneorum tricoccon*
408 (Cneoraceae), sexual expression and sex ratios varied seasonally over time and between
409 populations, indicating factors such as soil nutrients, light, precipitation, temperature, and
410 others as influencing the allocated sexual resources (Traveset, 1995). In *Solanum lycocarpum*,
411 we can infer that during the dry season, when resources are limited, the female function would
412 be prioritized up to the maximum fruit production supported by the individual, mainly due to
413 the energy cost of production of large fruits in the species. The fruit of *S. lycocarpum* is fleshy,
414 globose with a diameter ranging from 8 to 15 cm, weighs from 200 to 900 g, 600 to 800 seeds
415 per fruit and each plant produces on average 1.12 to 5.35 fruits (Lombardi & Motta-Junior,
416 1993; Martins, Chaves, Buso, & Kageyama, 2006; Moura et al., 2010). The energy cost of
417 production this fruit is so high that the hermaphrodite flowers of *S. lycocarpum* represent only
418 5% of total flower production (Moura et al. 2010), of which 91.7% formed fruit (Oliveira-Filho
419 & Oliveira, 1988). We found no evidence that female function is limited by floral visitors
420 because hermaphroditic flowers exposed to natural pollination and supplemental pollen did not
421 differ in fruit set. It is reasonable to think that when plants reach the limit of their fruit
422 production, the remaining resources can be converted into the production of staminate flowers.

423 Therefore, andromonoecy could be considered a reproductive strategy to take the best
424 advantage of environmental uncertainties through the male function, which are less
425 “expensive”. Differences in resource investment were found between staminate and
426 hermaphroditic flowers in *Capparis spinosa* (Capparaceae) (Zhang & Tan, 2009). In *S. hirtum*,
427 sexual expression was partially determined by the individual’s resource status and the female
428 function was prioritized (Diggle, 1993), while in *Solanum carolinense*, the environment has a
429 strong influence on the expression of plant and floral traits (Elle, 1999).

430

431 **Evolutionary implications of variation in the strength of andromonoecy among** 432 **individuals**

433 In the studied populations of *S. lycocarpum*, some individuals were exclusively male
434 (producing only staminate flowers) throughout the year. In individuals with both sexual
435 functions, there is a continuous variation in female function and there are no plants with only
436 hermaphrodite flowers in the populations. In *Tulipa pumila*, the presence of male individuals
437 may be the result of limited resources for reproduction, indicating that the only production of
438 staminate flowers may be a stage in growth to control sexual expression (Astuti, Pratesi, Carta,
439 & Peruzzi, 2020). In *S. lycocarpum* this can also occur, but further analysis is needed at this
440 point. In general, these populations behaved as “functionally androdioecious” within the studied
441 year. Andromonoecy in *S. lycocarpum* (as in other andromonoecious *Solanum*) is derived from
442 hermaphroditism, and staminate flowers have vestigial pistils (Coleman & Coleman, 1982;
443 Lewis, 1942; Zhang & Tan, 2009). On the other hand, the lower pollen viability in
444 hermaphroditic flowers may indicate specialization into female function. The evolutionary
445 significance of exclusively male individuals in the population of *S. lycocarpum* remains
446 unclear. On one hand, male-only individuals may represent an initial step towards the transition
447 to separate sexes (dioecy via the androdioecy pathway) (Primack & Lloyd, 1980; Solomon,
448 1986). Some species of the genus *Solanum* (such as *S. appendiculatum*, *S. asymmetriphyllum*,
449 *S. carduiforme*, *S. cataphractum*, *S. cunninghamii*, *S. dioicum*, *S. leopoldensis*, *S. petraeum*, *S.*
450 *tudununggae*, *S. vansittartensis*) are functionally dioecious: although they have staminate and
451 hermaphroditic flowers, the latter produce non-aperturate pollen grains that never germinate
452 (Anderson & Symon, 1989). It is believed that dioecy may have evolved from hermaphroditism
453 through andromonoecy, by means of an additional step towards androdioecy (Anderson &
454 Symon, 1989), mainly in species whose production of hermaphroditic flowers is low and fruits
455 production is absent in some individuals (Charlesworth, 1984), such as *Leptospermum*

456 *scoparium* (Myrtaceae) (Primack & Lloyd, 1980). On the other hand, the male-only individuals
457 that we observed here may produce hermaphroditic flowers at some point later during the
458 lifespan of this perennial species. Long-term surveys of wild populations of *S. lycocarpum* are
459 required to address this possibility.

460

461 **CONCLUSIONS**

462 In *Solanum lycocarpum*, staminate flowers were smaller and produced fewer pollen grains with
463 higher viability than hermaphroditic flowers. Although hermaphroditic flowers were larger,
464 visitors did not exhibit any preference for floral types. Sexual expression in *S. lycocarpum*
465 varied over time, with the production of staminate flowers being seasonal and that of
466 hermaphroditic flowers presenting no clear peak. This seasonality in the production of
467 staminate flowers is associated with the local climatic conditions. Thus, it can be inferred that
468 the andromonoecious sexual system can be variable and individuals can respond to
469 environmental variation by changing the relative production of staminate flowers. Further
470 studies on the production of floral types by *S. lycocarpum* in both natural and anthropized
471 environments will help to confirm our findings and contribute to explaining how sexual
472 expression is influenced by local environmental conditions. Sexually labile species, such as *S.*
473 *lycocarpum*, provide an excellent opportunity to study the ecology and evolution of plant
474 reproductive strategies.

475

476 **ACKNOWLEDGEMENTS**

477 This work was carried out with the support of the Coordenação de Aperfeiçoamento de
478 Pessoal de Nível Superior (CAPES) – Finance Code 001 - and the Minas Gerais Research
479 Funding Foundation (process FAPEMIG RED-00253-16). We thank the Programa de Pós-
480 Graduação em Biodiversidade Vegetal of the UFG for supporting this work; the members of
481 the Laboratório de Biologia Reprodutiva at UFCat for their assistance in data collection and
482 analysis; and Professor Dr. Antônio Aguiar for identifying the bees. We also thank Prof. Dr.
483 Jamir A. Prado Junior for collecting climatic data for the three populations studied here and two
484 anonymous reviewers for the invaluable contributions in the first version of the manuscript.

485

486 **REFERENCES**

- 487 Albal, A., Azad, G., Shrotri, S., & Gowda, V. (2020). When to be a male? Role of resource-
488 limitation and pollinators in determining gender in an andromonoecious spiderwort. *BioRxiv*.
489 doi:10.1101/2020.06.06.138354
- 490 Alvares, C.A., Stape, J.L., Sentelhas, P.C., Gonçalves, J.L.M.; & Sparovek, G. (2013).
491 Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, 22(6): 711-728.
492 doi:10.1127/0941-2948/2013/0507
- 493 Anderson, G.J., & Symon, D.E. (1989). Functional dioecy and andromonoecy in *Solanum*.
494 *Evolution*, 43(1), 204-219. doi: 10.1111/j.1558-5646.1989.tb04218.x
- 495 Ascheri, D.P.R., Moura, W.S., Ascheri, J.L.R., & Junior, E.A.F. (2009). Propriedades
496 termodinâmicas de adsorção de água do amido de rizomas do lírio-do-brejo (*Hedychium*
497 *coronarum*). *Ciência e Tecnologia de Alimentos*, 29(2), 1-9. doi:10.1590/S0101-
498 20612009000200036
- 499 Astuti, G, Pratesi, S, Carta, A, & Peruzzi, L. (2020). Male flowers in *Tulipa pumila* Moench
500 (Liliaceae) potentially originate from gender diphasy. *Plant Species Biol.*, 35, 130–137.
501 doi:10.1111/1442-1984.12267
- 502 Barrett, S.C.H., & Harder, L.D. (2006). David G. Lloyd and the evolution of floral biology:
503 from natural history to strategic analysis. In Harder, L.D., & Barrett, S.C.H. (Eds.), *Ecology*
504 *and Evolution of flowers*, Oxford University Press.
- 505 Bates, D., Mächler, M., Bolker, B.M., & Walker, S. (2015). Fitting linear mixed-effects models
506 using lme4. *Journal of Statistical Software*, 67(1), 1-48. doi:10.18637/jss.v067.i01
- 507 Bertin, R.I. (1982). The evolution and maintenance of andromonoecy. *Evolutionary Theory*, 6,
508 25-32.
- 509 Bohs, L. (2005). Major clades in *Solanum* based on *ndhF* sequences. In Keating, R.C.,
510 Hollowell V.C., & Croat, T.B. (Eds.), *A festschrift for William G. D'Arcy: the legacy of a*
511 *taxonomist*. (pp. 27-49). Monographs in Systematic Botany from the Missouri Botanical
512 Garden 104. Missouri Botanical Garden Press, St. Louis.
- 513 Calviño, A., Ashworth, L., & Moyetta, N. (2014). Fruit set increases with maleness in the
514 andromonoecious *Acacia caven*. *Flora*, 209, 457-463. doi: 10.1016/j.flora.2014.06.011

515 Cardoso, J.C.F., Viana, M.L., Matias, R., Furtado, M. T., Caetano, A.P.S., Consolaro, H., &
516 Brito, V.L.G. (2018). Towards a unified terminology for angiosperm reproductive systems.
517 *Acta Botanica Brasilica*, 32(3), 329-348. doi:10.1590/0102-33062018abb0124

518 Charlesworth, D. (1984). Androdioecy and the evolution of dioecy. *Biological Journal of the*
519 *Linnean Society*, 23, 333-348. doi:10.1111/j.1095-8312.1984.tb01683.x

520 Coleman, J.R., & Coleman, M.A. (1982). Reproductive biology of an andromonoecious
521 *Solanum* (*S. palinacanthum* Dunal). *Biotropica*, 14(1), 69-75. doi:10.2307/2387763

522 Diggle, P.K. (1991). Labile Sex Expression in Andromonoecious *Solanum hirtum*: Floral
523 Development and Sex Determination. *American Journal of Botany*, 78(3), 377-393.
524 doi:10.1002/j.1537-2197.1991.tb15200.x

525 Diggle, P.K. (1993). Developmental Plasticity, Genetic Variation, and the Evolution of
526 Andromonoecy in *Solanum hirtum* (Solanaceae). *American Journal of Botany*, 80(8), 967-973.
527 doi:10.2307/2445518

528 Diggle, P.K. (1994). The expression of andromonoecy in *Solanum hirtum* (Solanaceae):
529 phenotypic plasticity and antogenetic contingency. *American Journal of Botany*, 81(10), 1354-
530 1365. doi:10.2307/2445411

531 Ehrlén, J. (2015). Selection on flowering time in a life-cycle context. *Oikos*, 124, 92-101.
532 doi:10.1111/oik.01473

533 Elle, E. (1999). Sex allocation and reproductive success in the andromonoecious perennial
534 *Solanum carolinense* (Solanaceae). I. Female Success. *American Journal of Botany*, 86(2), 278-
535 286. doi:10.2307/2656944

536 Elle, E., & Meagher, T.R. (2000). Sex allocation and reproductive success in the
537 andromonoecious perennial *Solanum carolinense* (Solanaceae). II. Paternity and Functional
538 Gender. *The American Naturalist*, 156(6), 622-636. doi:10.1086/316997

539 Emms, S.K. (1993). Andromonoecy in *Zigadenus paniculatus* (Liliaceae): spatial and temporal
540 patterns of sex allocation. *American Journal of Botany*, 80(8), 914-923. doi:10.2307/2445511

541 Ferreira, F.S.O., & Cardoso, E. (2013). Estrutura fitossociológica de campo sujo no município
542 de Catalão - GO. *Caminhos de Geografia* 14(45), 110-119.

543 Fox, J., & Weisberg, S. (2011). *An R companion to applied regression*. Thousand Oaks: Sage.

544 Guimarães, L.S. (2008). *Mancha parda (Septoria glycines Hemmi) da soja (Glycine max L.):*
545 *aspectos etiológicos e de controle.* (Tese de Doutorado em Fitopatologia). Universidade de
546 Brasília, Brasília, DF.

547 Johansson, J., Bolmgren, K., & Jonzén, N. (2013). Climate change and the optimal flowering
548 time of annual plants in seasonal environments. *Global change biology*, *19*, 197-207.
549 doi:10.1111/gcb.12006

550 Klink, C.A., & Machado, R.B. (2005). A conservação do Cerrado brasileiro. *Megadiversidade*,
551 *1*(1), 147-155.

552 Köppen, W. (1948). *Climatologia: con un estudio de los climas de la tierra.* Fondo de Cultura
553 Econômica. México. 479p.

554 Krupnick, G.A., & Weis, A.E. (1998). Floral herbivore effect on the sex expression of an
555 andromonoecious plant, *Isomeris arborea* (Capparaceae). *Plant Ecology*, *134*, 151-162.
556 doi:10.1023/A:1009762415520

557 Kuhlmann, M. (2018). *Frutos e sementes do Cerrado: espécies atrativas para a fauna: volume*
558 *2.* Brasília: M. K. Peres. 464p.

559 Lewis, D. (1942). The evolution of sex in flowering plants. *Biological Reviews* *17*, 46-67.
560 doi:10.1111/j.1469-185X.1942.tb00431.x

561 Lloyd, D.G. (1979). Some reproductive factors affecting self-fertilization in angiosperms.
562 *American Naturalist*, *113*, 67-79. doi:10.1086/283365

563 Lloyd, D.G. (1980a). Sexual strategies in plants. I. An hypothesis of serial adjustment of
564 maternal investment during one reproductive session. *New Phytologist*, *86*, 69-79.
565 doi:10.1111/j.1469-8137.1980.tb00780.x

566 Lloyd, D.G. (1980b). Sexual strategies in plants. III. A quantitative method for describing the
567 gender of plants. *New Zealand Journal of Botany*, *18*, 103-108.
568 doi:10.1080/0028825X.1980.10427235

569 Lombardi, J.A., & Motta-Junior, J.C. (1993). Seed dispersal of *Solanum lycocarpum* St. Hil.
570 (Solanaceae) by the maned wolf, *Chrysocyon brachyurus* Illiger (Mammalia, Canidae). *Ciência*
571 *e Cultura*, *45*, 126-127.

572 Maêda, J.M. (1985). *Manual para uso da câmara de Newbauer para contagem de pólen em*
573 *espécies florestais*. Universidade Federal Rural do Rio de Janeiro, Departamento de
574 Silvicultura.

575 Martins, K., Chaves, L.J., Buso, G. S. C. & Kageyama, P. Y. (2006). Mating system and fine-
576 scale spatial genetic structure of *Solanum lycocarpum* St.Hil. (Solanaceae) in the Brazilian
577 Cerrado. *Conservation Genetics*, 7, 957-969. doi:10.1007/s10592-006-9140-y

578 Maruyama, P.K., Melo, C., Pascoal, C., Vicente, E., Cardoso, J.C.F., Brito, V.L.G., & Oliveira,
579 P.E. (2019). What is on the menu for frugivorous birds in the Cerrado? Fruiting phenology and
580 nutritional traits highlight the importance of habitat complementarity. *Acta Botanica Brasilica*,
581 33(3), 572-583. doi:10.1590/0102-33062019abb0221

582 May, P.G., & Spears-Jr., E.E. (1988). Andromonoecy and variation in phenotypic gender of
583 *Passiflora incarnata* (Passifloraceae). *American Journal of Botany*, 75 (12), 1830-1841.
584 doi:10.2307/2444737

585 McDonnell, A.J., Wetreich, H.B., Cantley, J.T., Jobson, P., & Martine, C.T. (2019). *Solanum*
586 *plastisexum*, an enigmatic new bush tomato from the Australian Monsoon Tropics exhibiting
587 breeding system fluidity. *PhytoKeys*, 124, 39–55. doi:10.3897/phytokeys.124.33526

588 Miller, J.S., & Diggle, P.K. (2003). Diversification of andromonoecy in *Solanum* section
589 *Lasiocarpa* (Solanaceae): the roles of phenotypic plasticity and architecture. *American Journal*
590 *of Botany*, 90(5), 707-715. doi:10.3732/ajb.90.5.707

591 Miller, J.S., & Diggle, P.K. (2007). Correlated evolution of fruit size and sexual expression in
592 andromonoecious *Solanum* sections *Acanthophora* and *Lasiocarpa* (Solanaceae). *American*
593 *Journal of Botany*, 94(10), 1706-1715. doi:10.3732/ajb.94.10.1706

594 Morellato, L.P.C., Alberti, L.F., & Hudson, I.L. (2010). Applications of circular statistics in
595 plant phenology: a case studies approach. In Hudson, I.L., & Keatley, M.R. (Eds.),
596 *Phenological Research* (pp. 339-359). Springer; Dordrecht, Netherlands. doi:10.1007/978-90-
597 481-3335-2_16

598 Morellato, L.P.C., Talora, D.C., Takahasi, A., Bencke, C.C., Romera, E.C., & Zipparro, V.B.
599 (2000). Phenology of Atlantic Rain Forest Trees: A Comparative Study. *Biotropica*, 32(4b),
600 811-823. doi:10.1111/j.1744-7429.2000.tb00620.x

601 Moura, T.M., Oliveira, G.C.X., & Chaves, L.J. (2010). Correlação entre floração, frutificação
602 e variáveis ambientais em *Solanum lycocarpum*. A. St. Hil., Solanaceae. *Bioscience Journal*,
603 26(3), 457-462.

604 NeSmith, D.S., Hoogenboom, G., & Groff, D.W. (1994). Staminate and Pistillate Flower
605 Production of Summer Squash in Response to Planting Date. *Hortscience*, 29(4), 256–257.
606 doi:10.21273/HORTSCI.29.4.256

607 Oliveira-Filho, A.T., & Oliveira, L.C.A. (1988). Biologia floral de uma população de *Solanum*
608 *lycocarpum* St. Hil. (Solanaceae) em Lavras-MG. *Revista Brasileira de Botânica*, 11, 23-32.

609 Peixoto, J.S.G. (2015). *A qualidade dos recursos hídricos superficiais no alto curso da bacia*
610 *hidrográfica do córrego Campo Alegre – Goiás*. (Dissertação de Mestrado em Geociências).
611 Universidade de Brasília, Brasília, DF.

612 Podolsky, R.D. (1992). Strange floral attractors: Pollinator attraction and the evolution of plant
613 sexual systems. *Science*, 258, 791-793. doi:10.1126/science.258.5083.791

614 Primack, R.B., & Lloyd, D.G. (1980). Andromonoecy in the New Zealand montane shrub
615 manuka, *Leptospermum scoparium* (Myrtaceae). *American Journal of Botany*, 67(3), 361-368.
616 doi:10.2307/2442346

617 Quesada-Aguilar, A., Kalisz, S., & Ashman, T.L. (2008). Flower morphology and pollinator
618 dynamics in *Solanum carolinense* (Solanaceae): implications for the evolution of
619 andromonoecy. *American Journal of Botany*, 95(8), 974-984. doi:10.3732/ajb.0800106

620 R Development Core Team. 2020. *R: A language and environment for statistical computing*. R
621 Foundation for Statistical Computing, Vienna, Austria. Url: <https://www.R-project.org/>.

622 Radford, A.E., Dickison, W.C., Massey, J.R., & Bell, C.R. (1974). *Vascular plant systematics*.
623 Harper e Row, New York, USA, 220 pp.

624 Rasband, W.S. (2016). *ImageJ*. National Institutes of Health, Bethesda, Maryland, USA, Url:
625 <<https://imagej.nih.gov/ij/>>

626 Richards, A.J. (1986). *Plant breeding systems*. Chapman Hall, London.

627 Reuther, K., & Bockhoff, R.C. (2013). Andromonoecy and developmental plasticity in
628 *Chaerophyllum bulbosum* (Apiaceae–Apioideae). *Annals of Botany*, 112, 1495-1503.
629 doi:10.1093/aob/mct073

630 Särkinen, T., Bohs, L., Olmstead, B.G., & Knaap, S. (2013). A phylogenetic framework for
631 evolutionary study of the nightshades (Solanaceae): a dated 1000-tip tree. *BMC Evolutionary*
632 *Biology*, 13(214), 1-15. doi:10.1186/1471-2148-13-214

633 Shwe, E., Wu, B., & Huang, S.Q. (2020). Both small and large plants are likely to produce
634 staminate (male) flowers in a hermaphrodite lily. *Plant diversity*. doi:10.1016/j.pld.2020.01.004

635 Solis-Montero, L., Vergara, C.H., & Vallejo-Marín, M. (2015). High incidence of pollen theft
636 in natural populations of a buzz-pollinated plant. *Arthropod-Plant Interactions*, 9, 599-611.
637 doi:10.1007/s11829-015-9397-5

638 Solis-Montero, L., & Vallejo-Marín, M. (2017). Does the morphological fit between flowers
639 and pollinators affect pollen deposition? An experimental test in a buzz-pollinated species with
640 anther dimorphism. *Ecology and Evolution*, 7, 2706-2715. doi:10.1002/ece3.2897

641 Solomon, B.P. (1985). Environmentally influenced changes in sex expression in an
642 andromonoecious plant. *Ecology*, 66(4), 1321-1332. doi:10.2307/1939185

643 Solomon, B.P. (1986). Sexual allocation and andromonoecy: resource investment in male and
644 hermaphrodite flowers of *Solanum carolinense* (Solanaceae). *American Journal of Botany*,
645 73(8), 1215-1221. doi:10.1002/j.1537-2197.1986.tb08568.x

646 Spalik, K. (1991). On evolution of andromonoecy and ‘overproduction’ of flowers: a resource
647 allocation model. *Biological Journal of the Linnean Society*, 42, 325-336. doi:10.1111/j.1095-
648 8312.1991.tb00566.x

649 Symon, D.E. (1979). Sex forms in *Solanum* (Solanaceae) and the role of pollen collecting
650 insects. In Hawkes, J.G., Lester, R.N., & Skelding, A.D. (Eds.), *The biology and taxonomy of*
651 *the Solanaceae* (pp. 385-397). Academic Press, London, UK.

652 Tavares, P.R.A., Alves-Junior, V.V., Morais, G.A., Polatto, L.P., & Dutra, J.C.S. (2018). Pollen
653 availability and behavior of visiting bees of *Solanum lycocarpum* A. St. Hill (Solanaceae).
654 *Entomological News*, 127(4), 375-385. doi:10.3157/021.127.0410

655 Tavares, P.R.A., Alves-Junior, V.V., Morais, G.A., & Polatto, L.P. (2017). A interrelação entre
656 a morfologia floral de *Solanum lycocarpum* e o tamanho corporal das abelhas visitantes garante
657 o sucesso reprodutivo? *Interciencia*, 42(6), 375-379.

658 Toji, T., & Itino, T. (2020). Differences in sex expression and mating systems in three
659 pollination morphs of *Cimicifuga simplex*. *Plant Species Biol.* 35, 112– 119. doi:10.1111/1442-
660 1984.12265

661 Traveset, A. (1995). Reproductive ecology of *Cneorum tricoccon* L. (Cneoraceae) in the
662 Balearic Islands. *Botanical Journal of the Linnean Society*, 117, 221-232. doi:10.1111/j.1095-
663 8339.1995.tb00453.x

664 Vallejo-Marín, M. (2019). Buzz pollination: studying bee vibrations on flowers. *New*
665 *Phytologist*, 224(3), 1068-1074. doi:10.1111/nph.15666

666 Vallejo-Marín, M., & Rausher, M.D. (2007a). Selection through female fitness helps to explain
667 the maintenance of male flowers. *The American Naturalist*, 169(5), 563-568.
668 doi:10.2307/4137036

669 Vallejo-Marín, M., & Rausher, M.D. (2007b). The role of male flowers in andromonoecious
670 species: energetic costs and siring success in *Solanum carolinense* L. *Evolution*, 61(2), 404-
671 412. doi:10.1111/j.1558-5646.2007.00031.x

672 Whalen, M.D., & Costish, D.E. (1986). Andromonoecy in *Solanum*. In: D'Arcy, W.G. (Ed.)
673 *Solanaceae: Biology and systematics*. Columbia University Press, New York, p. 284-302.

674 Yampolsky, C., & Yampolsky, H. (1922). Distribution of sex forms in the phanerogamic flora.
675 *Bibliotheca Genetica*, 3, 1-62.

676 Zar, J.H. (2010). *Biostatistical analysis*. Prince-Hall International, Upper Saddle River, New
677 Jersey, USA: 960 pp.

678 Zhang, T., & Tan, D.Y. (2009). An examination of the function of male flowers in an
679 andromonoecious shrub *Capparis spinosa*. *Journal of Integrative Plant Biology*, 51(3), 316-
680 324. doi:10.1111/j.1744-7909.2008.00800.x

681 **TABLES**

682 **Table 1.** Flower traits of *Solanum lycocarpum* A. St. -Hil. (Solanaceae) compared between
 683 floral types (hermaphroditic and staminate) and populations (UFG Farm, Catalão, Goiás,
 684 Brazil; Campo Alegre de Goiás, Goiás, Brazil; and Pontinhas Farm, Orizona, Goiás, Brazil).
 685 ANOVA was used for the analysis. F-values, degrees of freedom (df), and p-values are
 686 presented, as well as overall means in mm, standard deviations (SD), and sample number (N)
 687 of hermaphroditic and staminate flowers. *Significant values.

Variables	ANOVA			Mean \pm SD (N)	
	df	F	p	Hermaphroditic	Staminate
Corolla length				56.35 \pm 0.35 (45)	47.65 \pm 3.02 (45)
Floral type	1	27.74	< 0.001*		
Population	1	1.13	0.29		
Floral type -population interaction	1	1.84	0.18		
Corolla width				49.98 \pm 1.92 (45)	42.33 \pm 1.20 (45)
Floral type	1	16.73	< 0.001*		
Population	1	0.09	0.76		
Floral type -population interaction	1	0.38	0.53		
Anther length				17.63 \pm 0.29 (45)	17.86 \pm 0.96 (45)
Floral type	1	0.26	0.61		
Population	1	0.92	0.34		
Floral type -population interaction	1	2.83	0.09		
Anther width				2.29 \pm 0.11 (45)	2.09 \pm 0.09 (45)
Floral type	1	6.63	0.01*		
Population	1	1.55	0.21		
Floral type -population interaction	1	0.78	0.37		
Style length				21.14 \pm 0.73 (45)	2.50 \pm 0.23 (45)
Floral type	1	1684.76	< 0.001*		
Population	1	0.13	0.71		
Floral type -population interaction	1	0.08	0.77		

688

Table 2. Quantity and viability of pollen and ovules in hermaphroditic and staminate flowers of *Solanum lycocarpum* A. St. -Hil. (Solanaceae) in three populations (UFG Farm, Catalão, Goiás, Brazil; Campo Alegre de Goiás, Goiás, Brazil; and Pontinhas Farm, Orizona, Goiás, Brazil). Data were compared between floral types, populations, and floral types within populations (interaction between population and floral type) using a GLMM; the degrees of freedom (df), Chi-square (χ^2) and p values are presented. *Significant values. Mean and standard deviations (SD) of the variables in hermaphroditic and staminate flowers and number of flowers evaluated (N).

Variables	GLMM			Mean \pm SD(N)	
	d f	χ^2	p	Hermaphroditic	Staminate
Pollen quantity				$1.85 \times 10^6 \pm 0.87 \times 10^6$ (45)	$1.74 \times 10^6 \pm 0.71 \times 10^6$ (45)
Floral type	1	23212	< 0.001*		
Population	2	388942	< 0.001*		
Floral type - population interaction	2	12279	< 0.001*		
Pollen viability				$56 \pm 33\%$ (45)	$78 \pm 22\%$ (45)
Floral type	1	127.32	< 0.001*		
Population	2	8.98	0.01*		
Floral type - population interaction	2	7.48	0.02*		
Number of ovules				395 ± 99 (30)	141 ± 32 (30)
Floral type	1	896.71	< 0.001*		
Population	2	29.77	< 0.001*		
Floral type - population interaction	2	23.66	< 0.001*		

Table 3. Results of the mating system tests conducted on floral types (hermaphroditic and staminate) in Pontinhas Farm, Orizona, Goiás, Brazil. Equal letters indicate lack of evidence that Cross- and Natural pollination produce different fruit set in the population under a Chi-square test.

Treatments	Fruits obtained (tested flowers) – Percentage of success	
	Staminate flowers	Hermaphroditic flowers
Cross-pollination	0 (15) – 0%	10 (15) – 67% ^a
Natural pollination	0 (15) – 0%	8 (15) – 54% ^a
Manual self-pollination	0 (15) – 0%	0 (15) – 0%
Spontaneous self-pollination	0 (15) – 0%	0 (15) – 0%
Agamospermy	0 (15) – 0%	0 (15) – 0%

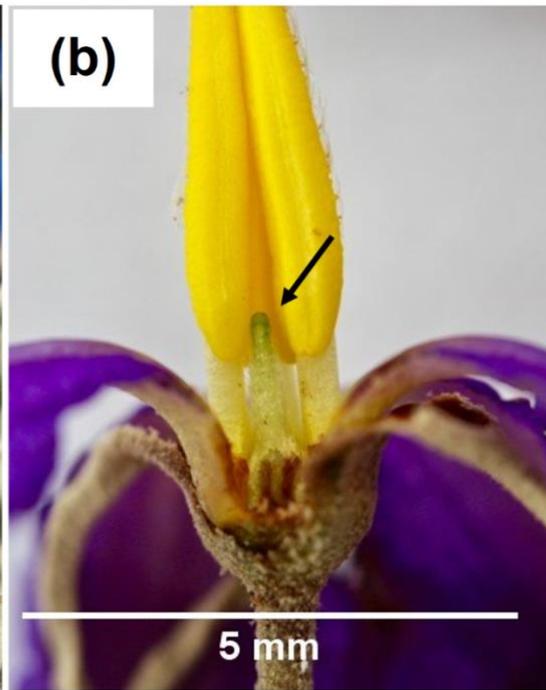
FIGURES LEGENDS

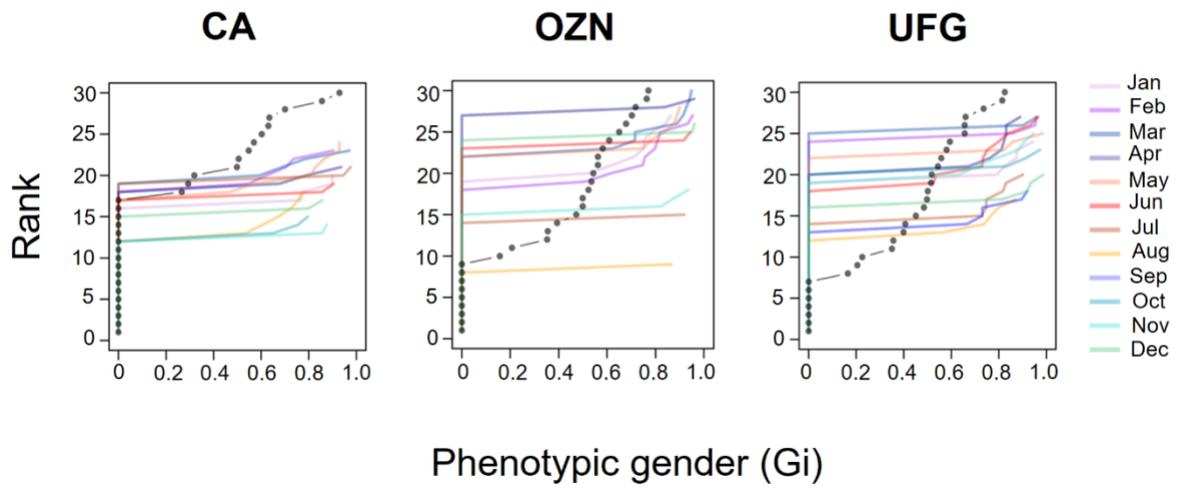
Figure 1. Flowers of *Solanum lycocarpum* A. St. -Hil. (Solanaceae) and their frequent visitors in UFG Farm, Catalão, Goiás, Brazil. (a) Hermaphroditic flower, with arrow indicating the stigma projecting above the anther cone, (b) Staminate flower, with arrow indicating the pistiloid, (c) *Exomalopsis fulvofasciata* visiting staminate/hermaphroditic flowers and (d) *Augochloropsis* sp. visiting a staminate/hermaphroditic flower.

Figure 2. Variations in phenotypic gender in *Solanum lycocarpum* A. St. -Hil. (Solanaceae) from January to December 2017 in three populations in the Brazilian Cerrado. Warm colors lines represent dry months while cool colors lines represent humid months. Each line represents a month and the corresponding colors and months are in the legend. Black dots and lines represent the phenotypic gender considering the total flower production along the year. *Gi* values can vary from 0 to 1, which represents strictly male and female plants, respectively. Rank values represents each plant in each month. CA – Campo Alegre de Goiás, Goiás, Brazil; OZN – Pontinhas Farm, Orizona, Goiás, Brazil; UFG – UFG Farm, Catalão, Goiás, Brazil.

Figure 3. Mean production of (a) staminate and (b) hermaphroditic flowers, as well as (c) fruits of *Solanum lycocarpum* A. St. -Hil. (Solanaceae) per individual along the year (numeric bar) in three populations (UFG Farm, Catalão, Goiás, Brazil; Campo Alegre de Goiás, Goiás, Brazil; and Pontinhas Farm, Orizona, Goiás, Brazil). Blue is the humid months and red is the dry months.

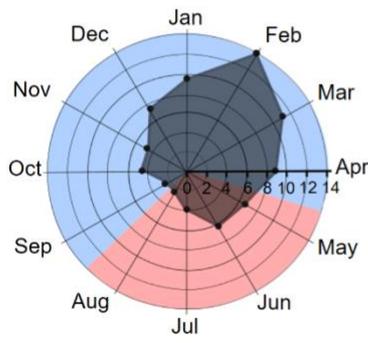
Figure 4. Number of staminate flowers produced per plant depending on the climatic condition (dry or humid) in *Solanum lycocarpum* A. St. -Hil. (Solanaceae). In the boxplots, dark lines indicate the medians, boxes represent second and third quartiles and whiskers represent first and fourth quartiles. Black dots are outliers; gray dots are the real data.



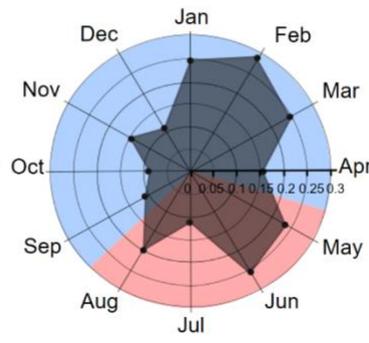


Mean production per individual

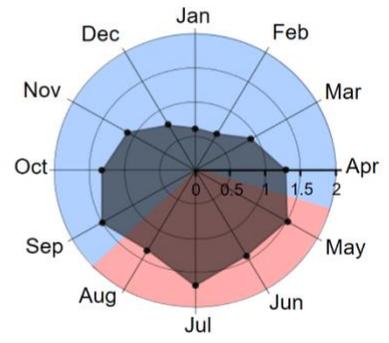
a

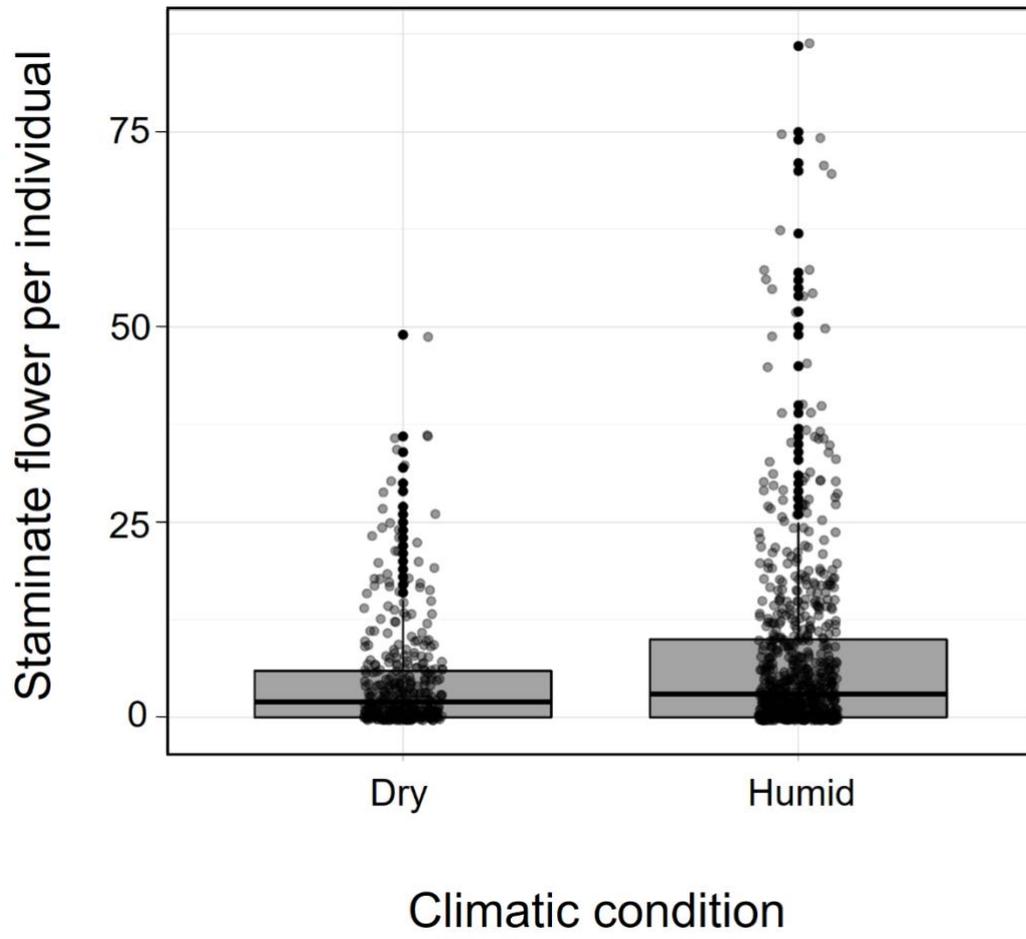


b

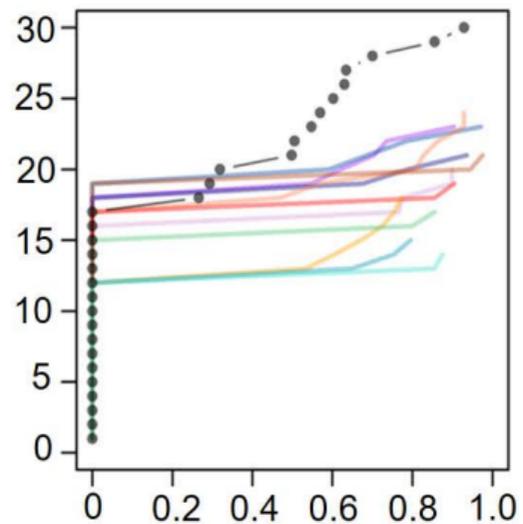
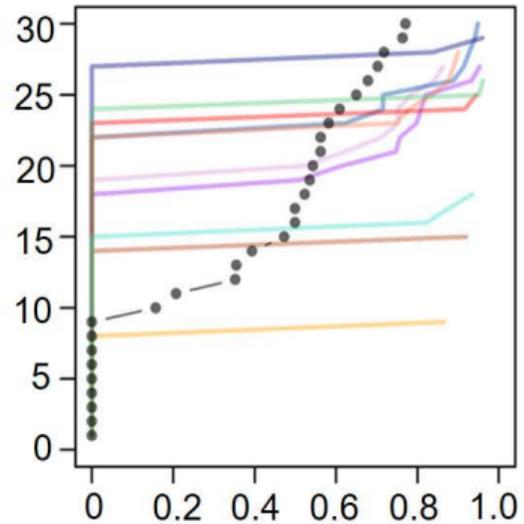
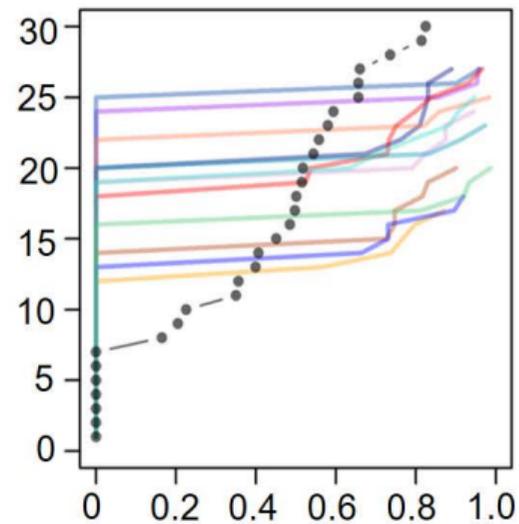


c







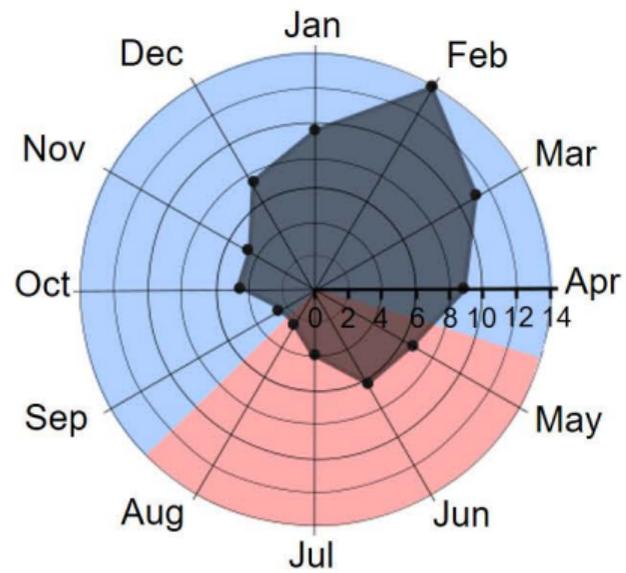
CA**OZN****UFG**

Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec

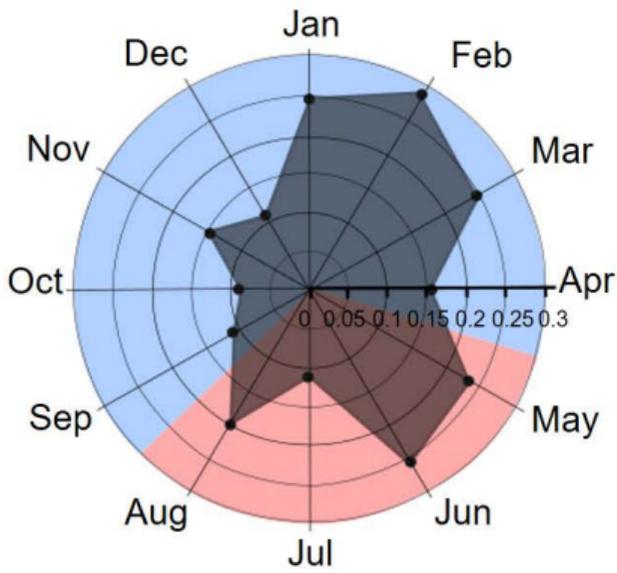
Phenotypic gender (G_i)

Mean production per individual

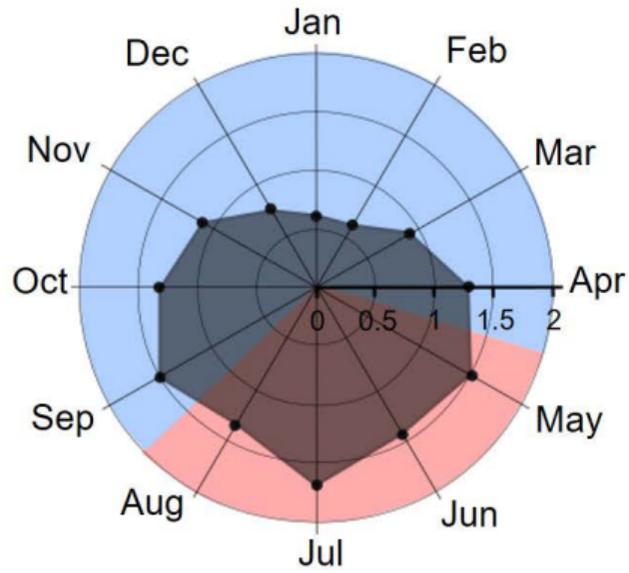
a



b

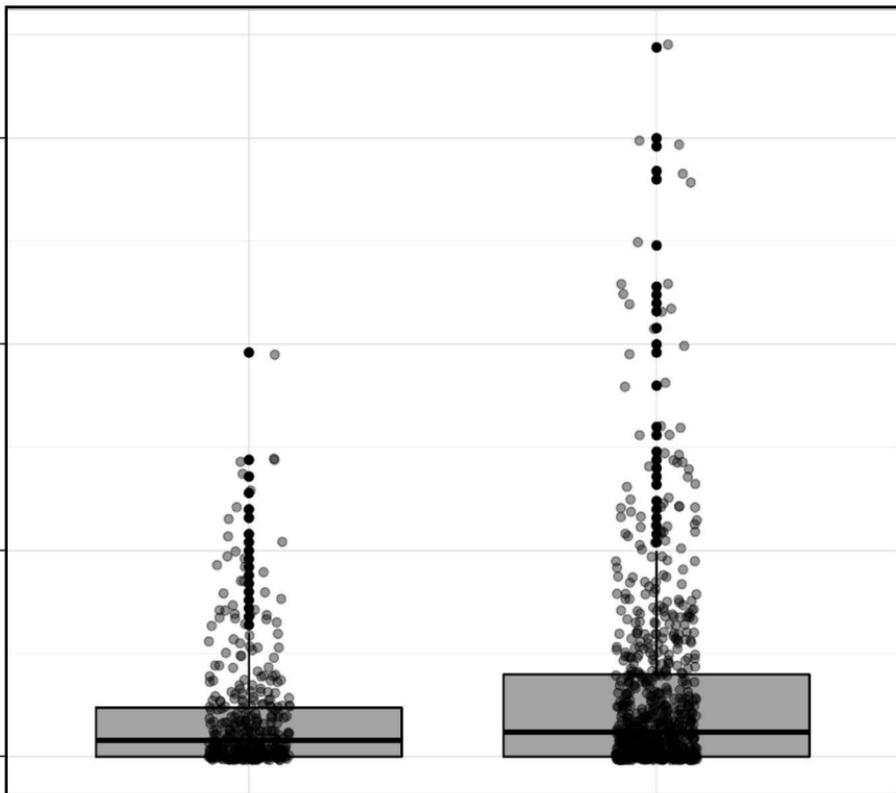


c



Staminate flower per individual

75
50
25
0



Dry

Humid

Climatic condition

SUPPORTING INFORMATION

Table S1. Measures of length and width of the thorax, length and width of the abdomen, and total length of bee species observed visiting flowers of *Solanum lycocarpum* A. St. -Hil.. Number of visits observed by bee specimens (grouped in genera) and the percentage of visits (number of visits of the genera / total of visits in the population) in three populations (UFG Farm, Catalão, Goiás, Brazil (UFG); Campo Alegre de Goiás, Goiás, Brazil (CA); and Pontinhas Farm, Orizona, Goiás, Brazil (OZN)). In the last lines are the means in mm and standard deviations (SD) of the traits measured and the total number of visits in each population.

Bee species	Measures in mm					Number of visits (Percentage)		
	Length of the thorax	Width of the thorax	Length of the abdomen	Width of abdomen	Total length	Population		
						UFG	CA	OZN
<i>Augochloropsis</i> sp. 1 (Cockerell, 1897)	2.72	2.62	4.34	2.53	10.12	30 (25%)	24 (28%)	8 (13%)
<i>Augochloropsis</i> sp. 2 (Cockerell, 1897)	2.34	2.41	3.17	2.24	8.19			
<i>Bombus morio</i> (Swederus, 1787)	5.49	7.32	7.80	8.54	16.95			
<i>Bombus brevivillus</i> (Franklin 1913)	5.22	6.23	6.46	8.83	15.67	21 (17%)	0	1 (2%)
<i>Bombus pauloensis</i> (Friese, 1913)	5.01	6.56	6.88	7.19	14.80			
<i>Centris scopipes</i> (Friese, 1899)	10.56	12.06	12.55	13.30	29.86	3 (2%)	2 (2%)	18 (29%)
<i>Epicharis analis</i> (Lepeletier, 1841)	6.23	6.36	7.42	9.53	20.12			
<i>Epicharis flava</i> (Friese, 1900)	8.71	7.63	7.82	9.31	23.78	34 (28%)	6 (7%)	6 (10%)

Bee species	Measures in mm					Number of visits (Percentage)		
	Length of the thorax	Width of the thorax	Length of the abdomen	Width of abdomen	Total length	Population		
						UFG	CA	OZN
<i>Exomalopsis analis</i> (Spinola, 1853)	2.32	2.37	3.08	2.84	7.38			
<i>Exomalopsis collaris</i> (Friese, 1899)	2.30	3.43	4.22	3.33	10.46	8 (7%)	21 (25%)	7 (11%)
<i>Exomalopsis fulvofasciata</i> (Smith, 1879)	3.46	4.26	5.38	4.61	11.16			
<i>Melipona quinquefasciata</i> (Lepeletier, 1835)	3.54	3.95	4.17	3.76	10.56	4 (3%)	2 (2%)	1 (2%)
<i>Oxaea cf. flavescens</i> (Klung, 1807)	5.59	7.24	7.61	7.51	18.00	20 (16%)	30 (35%)	10 (16%)
<i>Paratetrapedia lugubris</i> (Cresson, 1878)	3.76	3.90	5.84	4.11	13.34	2 (2%)	0	0
<i>Xylocopa suspecta</i> (Moure & Camargo, 1988)	9.27	10.38	9.50	10.13	23.26	0	0	11 (18%)
<i>Xylocopa subcyanea</i> (Perez, 1901)	6.75	6.87	8.79	9.40	18.33			
Mean ± SD	5.21 ± 2.59	5.85 ± 2.81	6.56 ± 2.53	6.70 ± 3.36	15.75 ± 6.29	-	-	-
Total number of visits	-	-	-	-	-	122	85	62

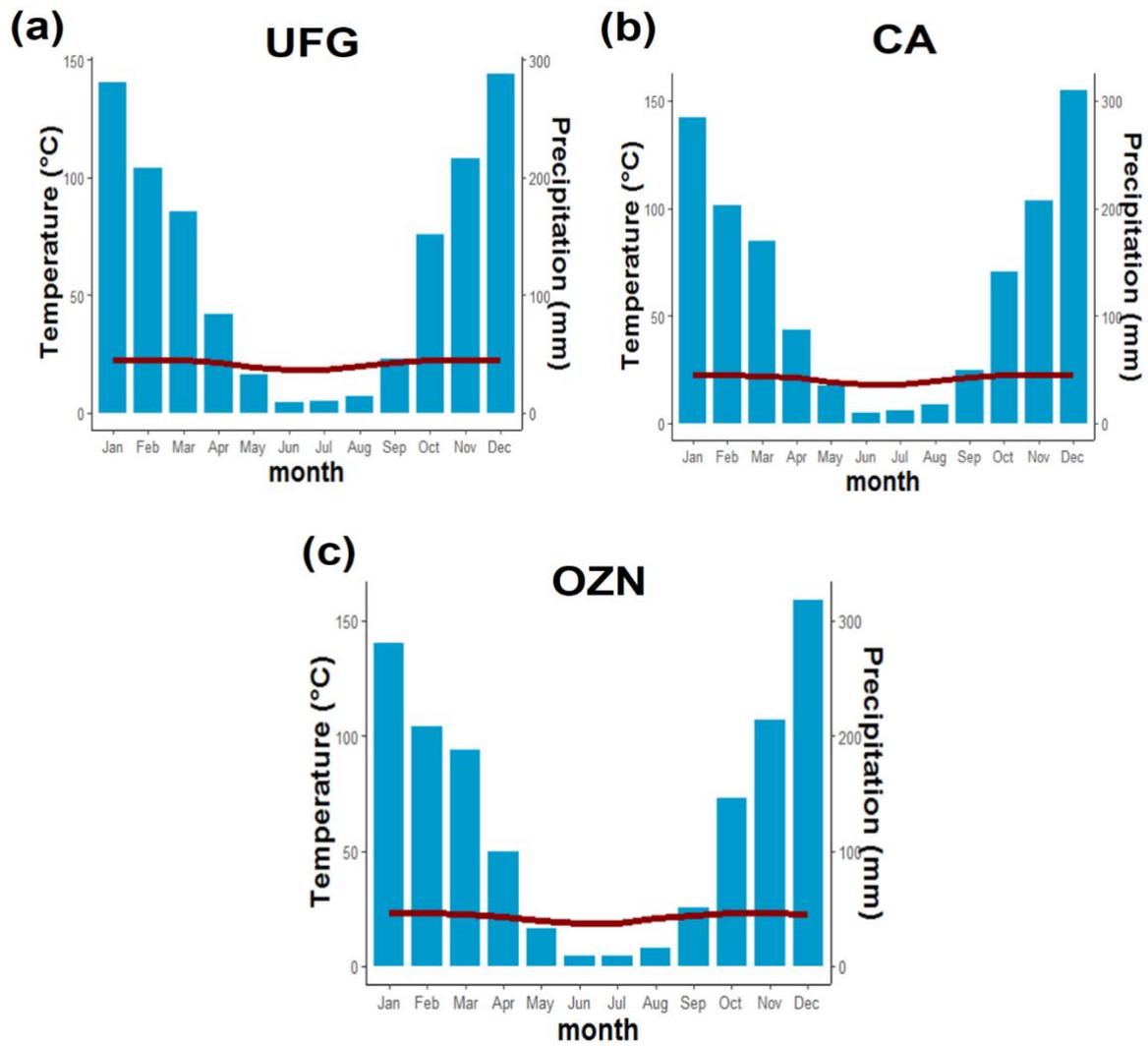


Figure S1. Classification of dry and humid months based on climate graphs. The monthly values correspond to the average temperature and precipitation between 1950-1990. In dry months, the blue bars of precipitation are below the red line of temperature, while in humid months, the bars are above the lines. (a) UFG Farm, Catalão, GO (UFG); (b) Campo Alegre de Goiás, GO (CA); and (c) Pontinhas Farm, Orizona, GO (OZN).

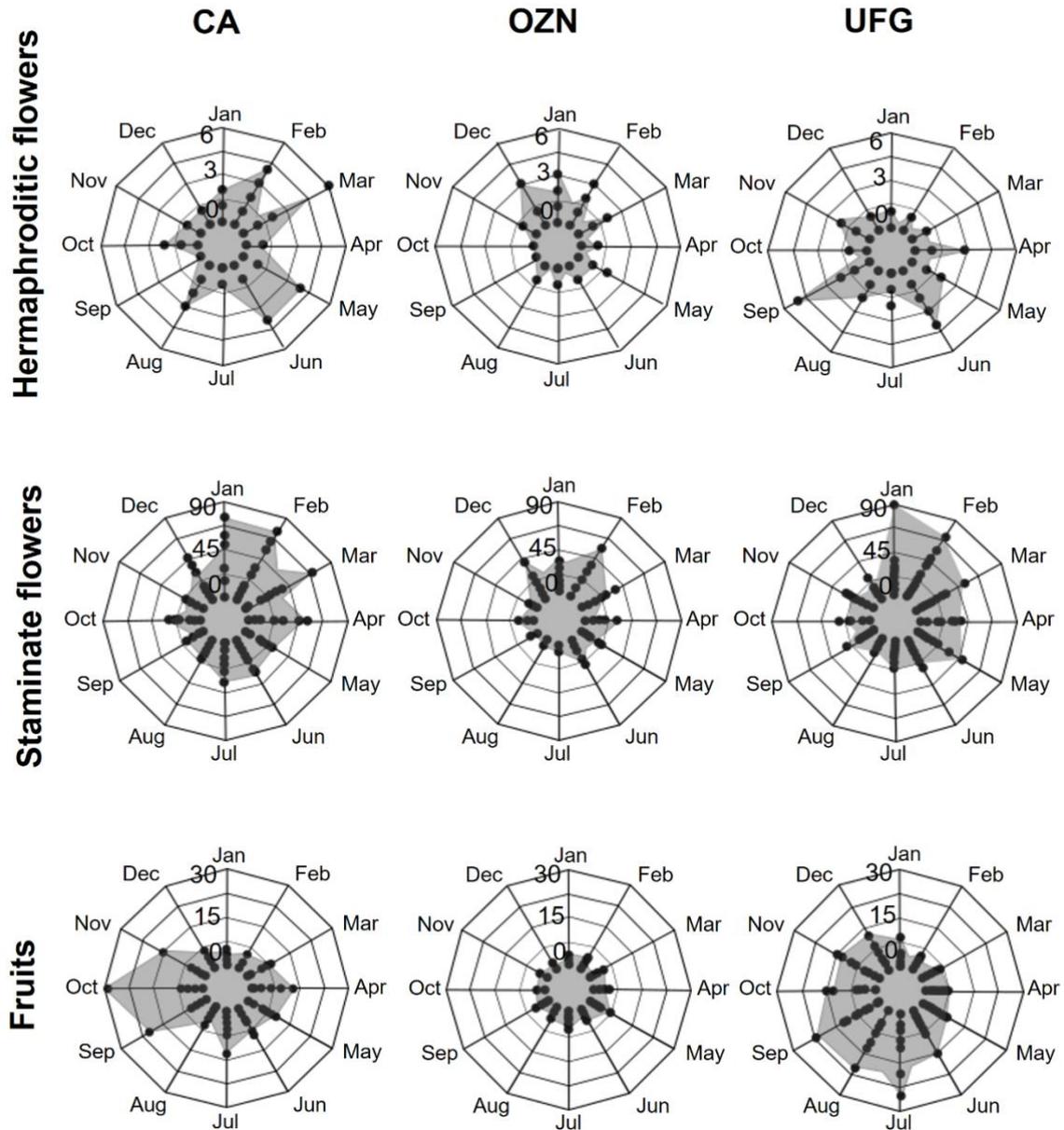
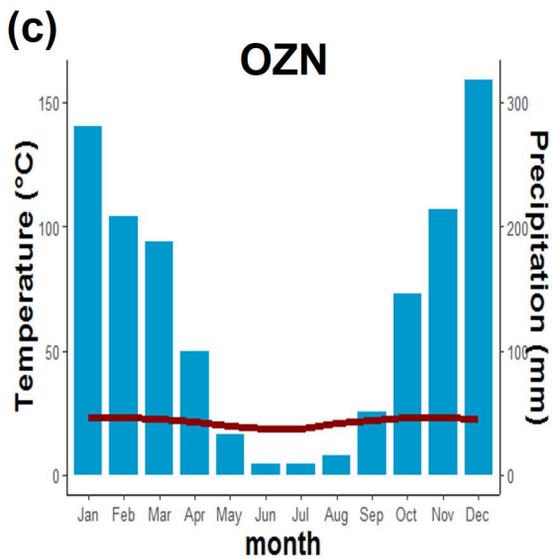
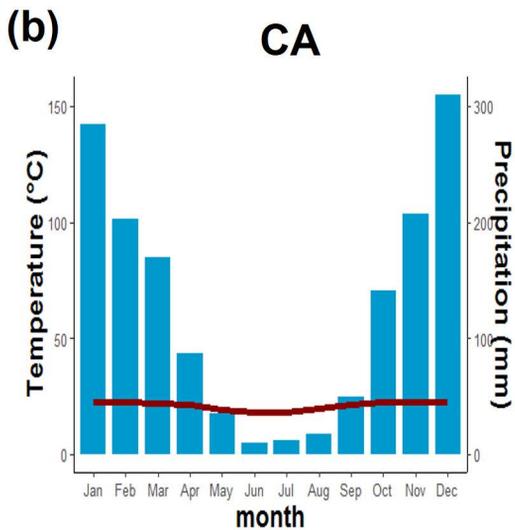
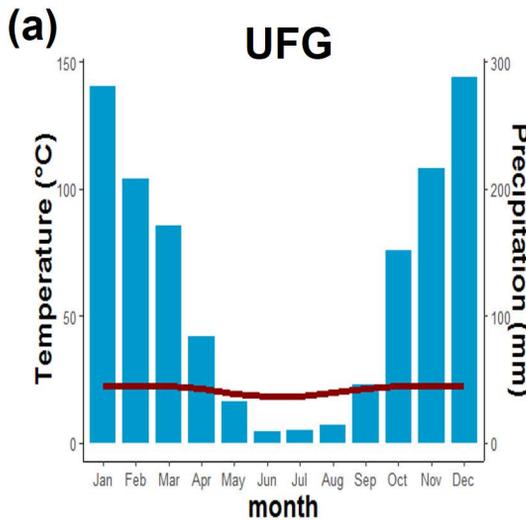
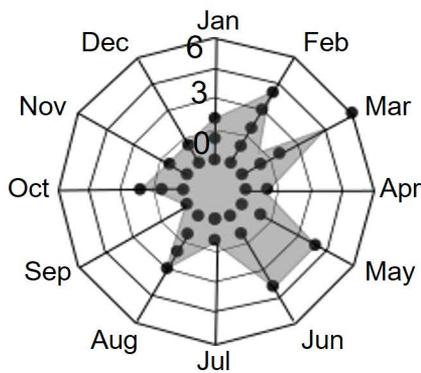


Figure S2: Production of both hermaphroditic and staminate flowers, as well as fruit production per individual of *Solanum lycocarpum* A. St. Hil. (Solanaceae) along the year (numeric bar) in each population (CA: Campo Alegre de Goiás- GO; OZN: Pontinhas Farm, Orizona- GO; UFG: UFG Farm, Catalão, GO).

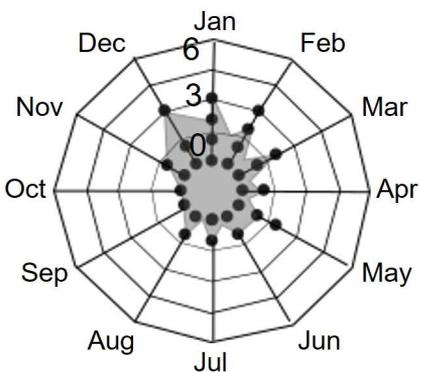


Hermaphroditic flowers

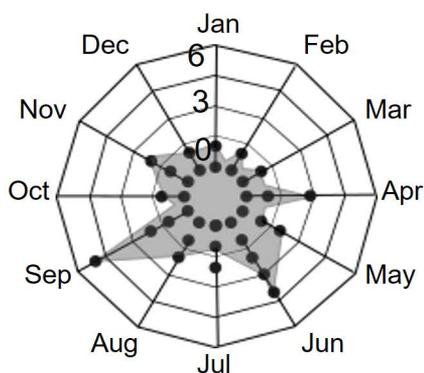
CA



OZN

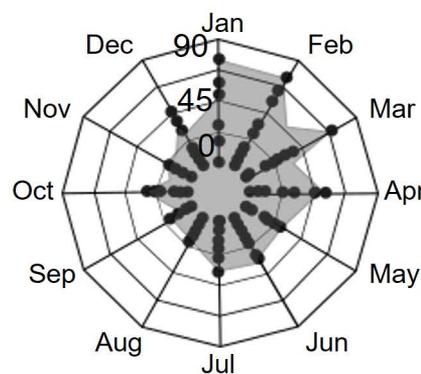


UFG

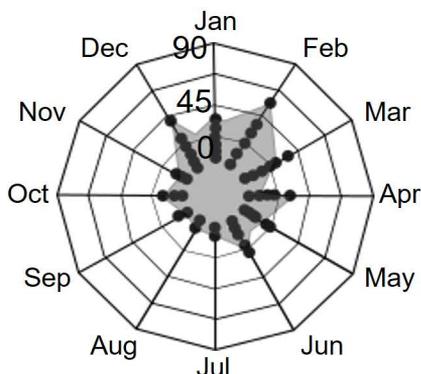


Staminate flowers

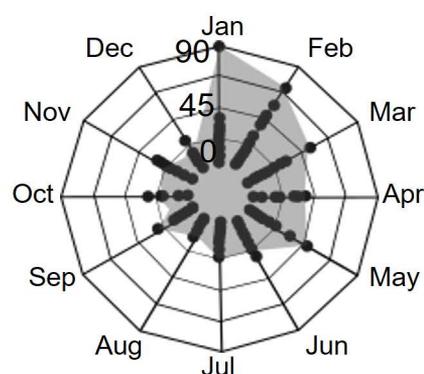
CA



OZN

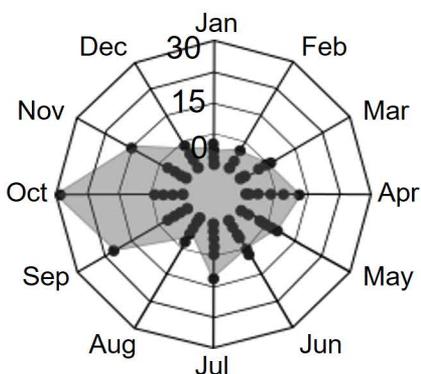


UFG

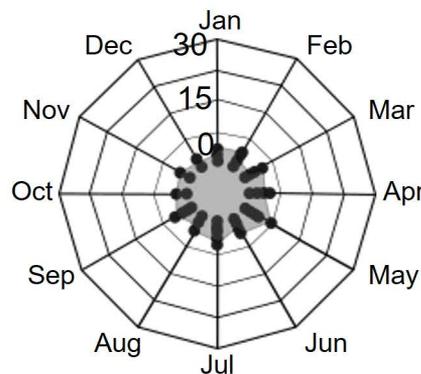


Fruits

CA



OZN



UFG

