ORIGINAL ARTICLE

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

Vanessa Gonzaga Marcelo[1], Vinícius Lourenço Garcia de Brito[2], Mario Vallejo-Marín[3], Hélder Consolaro[4]

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

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

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

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

*Corresponding author: vanessamarcelo.bio@gmail.com

Author's present address: Programa de Pós-Graduação em Ecologia e Conservação de Recursos Naturais, Universidade Federal de Uberlândia, 38400-902, Uberlândia, Minas Gerais, Brazil

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

Keywords: Buzz pollination, Cerrado, reproductive biology, sexual expression, sexual system.

INTRODUCTION

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

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

Much less is known about the extent to which individual populations vary in the sexual expression over time. Most studies with andromonoecious populations characterize only a single sampling event, and thus do not allow evaluating temporal variation of individual populations. We expect sexual expression to be sensitive to temporal variations in environmental conditions (Calviño, Ashworth, & Moyetta, 2014; Diggle, 1993; Solomon, 1985) and thus might vary both within and among seasons. As plant phenological responses such as flowering and fruiting are influenced by present and past local climatic variation (Ehrlén, 2015; Johansson, Bolmgren, & Jonzén, 2013), it is expected that hermaphroditic and staminate flower production vary along the year, especially in seasonal environments. This variation would ultimately affect the phenotypic gender of the plants (i.e. the relative contribution of individuals to the next generation as female and male parents; Lloyd, 1979). Moreover, since hermaphroditic flowers are considered more costly to produce than staminate flowers and staminate flowers are more susceptible to the climatic conditions over time (NeSmith, Hoogenboom, & Groff 1994). Therefore, it is expected that staminate flower production is more sensitive to variation in local climatic conditions than the production of hermaphroditic flowers in andromonoecious plants. Such spatial and temporal dynamic of the maleness expression of each individual is interpreted as a mechanism of cost savings in investments and optimization of fruit development (Reuther & Bockhoff, 2013).

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

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

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

MATERIALS AND METHODS

Study area

Three populations in central Brazil were selected for monitoring. The region where the three areas are located has two defined seasons, a rainy summer (September to April) and a dry winter (May to August) (Köppen, 1948, Alvares et al., 2013; Maruyama et al., 2019; Figure S1), and average annual rainfall of 1,500 mm (Klink & Machado, 2005). The first population (UFG) is in Pé-do-Morro Farm (Federal University of Catalão, Catalão, Goiás; 47°59’55.6” W and 18°06’51.9” S), at an elevation of 830 m a.s.l. and with average temperature always above 18 ºC (Ferreira & Cardoso, 2013). The second population (CA) is located in a rural area of the municipality of Campo Alegre de Goiás, Goiás, (47º62’48.37” W and 17º76’09.34” S) at 760 m a.s.l. and average annual temperature of 20 ºC (Peixoto, 2015). The third population (OZN) is located on Pontinhas Farm, located ten kilometers from the municipality of Orizona, Goiás (48°23’30.9” W and 16°98’10.9” S), at 860 m a.s.l. and with an average temperature of 22 ºC (Ascheri, Moura, Ascheri, & Junior, 2009; Guimarães, 2008). As *S. lycocarpum* is very common in these areas, samples previously collected from these areas and deposited in the UFG herbarium (UFG 24361. Rizzo, J.A. et al., 13058 in 2005) and in Embrapa herbarium (CEN 105156. Rocha, G.P.E. 441 in 2015), were used as vouchers for this work.

Floral biology

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

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

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

Mating system

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

Pollination biology

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

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

Temporal variation in sexuality

Thirty individuals in each population were marked and monitored monthly from January to December 2017, totaling 90 individuals in the three populations. In the flowering and fruiting phenophases, the presence of open flowers and the presence and quantity of fruits were evaluated. In addition to phenological monitoring, the number of hermaphroditic and staminate flowers (floral types) was counted. Visual identification of floral types was easy because hermaphroditic flowers have a well-developed pistil, while staminate flowers have a reduced pistil. Afterwards, the number of each floral type and the estimated number of viable pollen grains and functional ovules produced by each floral type was used to calculate the phenotypic gender (*G*) of each plant by the following equation (Barrett & Harder, 2006, Lloyd, 1980b):

$$G = \frac{di}{di + li\,.\frac{\sum_i \quad di}{\sum_i \quad li}}$$

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

Statistical analysis

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

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

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

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

RESULTS

Floral biology

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

Hermaphroditic flowers have stamens and a long pistil (Figure 1a), while staminate flowers also have stamens but a reduced pistil (pistillode) (Figure 1b). Moreover, hermaphroditic flowers are usually located at the base of the inflorescence, being the first to open. The length ($F = 27.74$, $df = 1$, $p < 0.001$) and width ($F = 16.73$, $df = 1$, $p < 0.001$) of the corolla of hermaphroditic flowers were greater than those of staminate flowers (Table 1). Anther length did not differ between floral types (F = 0.26, df = 1, p = 0.61) (Table 1), but anther width was greater in hermaphroditic flowers (F = 6.63, df = 1, p = 0.01) (Table 1). Staminate flowers have a reduced pistil, so that the style and stigma do not project beyond the cone of anthers (Figure 1b); in turn, the style and stigma in hermaphroditic flowers are exerted by about four millimeters above the anther cone (Figure 1a). Thus, the style is longer in hermaphroditic than in staminate flowers (F = 1684.76, df = 1, $p < 0.001$). There was no difference in floral attributes among populations (Table 1).

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

Mating system

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

Pollination biology

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

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

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

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

Temporal variation in sexuality

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

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

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

DISCUSSION

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

Floral and pollination biology

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

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

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

Temporal variation in sexuality

Andromonoecy is likely a mechanism of resource allocation in which staminate flowers, with their reduced ovaries and lower energy investment in the production of smaller petals, save and distribute energy to be redistributed to other functions in the plant, such as vegetative growth, storage, and/or increased reproductive potential (Bertin, 1982; Elle, 1999; Emms, 1993; Miller & Diggle, 2007; Primack & Lloyd, 1980; Solomon, 1986; Spalik, 1991). If resources are unpredictable in previous stages, the ability to regulate the investment during the fruiting stage must be advantageous, because fruit development represents a significant drain of the available resources (Lloyd, 1980a). Thus, if non-production of viable pistils and ovules in staminate flowers is related to the allocation of the available resources, species with large fruits should be more strongly andromonoecious than those with small fruits, since more resources are needed for fruit maturation (Miller & Diggle, 2003; Symon, 1979).

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

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

Evolutionary implications of variation in the strength of andromonoecy among individuals

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

CONCLUSIONS

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

ACKNOWLEDGEMENTS

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

REFERENCES

Albal, A., Azad, G., Shrotri, S., & Gowda, V. (2020). When to be a male? Role of resource-limitation and pollinators in determining gender in an andromonoecious spiderwort. *BioRxiv*. doi:10.1101/2020.06.06.138354

Alvares, C.A., Stape, J.L., Sentelhas, P.C., Gonçalves, J.L.M.; & Sparovek, G. (2013). Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, *22*(6): 711-728. doi:10.1127/0941-2948/2013/0507

Anderson, G.J., & Symon, D.E. (1989). Functional dioecy and andromonoecy in *Solanum*. *Evolution, 43*(1), 204-219. doi: 10.1111/j.1558-5646.1989.tb04218.x

Ascheri, D.P.R., Moura, W.S., Ascheri, J.L.R., & Junior, E.A.F. (2009). Propriedades termodinâmicas de adsorção de água do amido de rizomas do lírio-do-brejo (*Hedychium coronarium*). *Ciência e Tecnologia de Alimentos, 29*(2), 1-9. doi:10.1590/S0101-20612009000200036

Astuti, G, Pratesi, S, Carta, A, & Peruzzi, L. (2020). Male flowers in *Tulipa pumila* Moench (Liliaceae) potentially originate from gender diphasy. *Plant Species Biol., 35,* 130–137. doi:10.1111/1442-1984.12267

Barrett, S.C.H., & Harder, L.D. (2006). David G. Lloyd and the evolution of floral biology: from natural history to strategic analysis. In Harder, L.D., & Barrett, S.C.H. (Eds.), *Ecology and Evolution of flowers*, Oxford University Press.

Bates, D., Mächler, M., Bolker, B.M., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1-48. doi:10.18637/jss.v067.i01

Bertin, R.I. (1982). The evolution and maintenance of andromonoecy. *Evolutionary Theory, 6*, 25-32.

Bohs, L. (2005). Major clades in *Solanum* based on *ndhF* sequences. In Keating, R.C., Hollowell V.C., & Croat, T.B. (Eds.), *A festschrift for William G. D'Arcy*: the legacy of a taxonomist. (pp. 27-49). Monographs in Systematic Botany from the Missouri Botanical Garden 104. Missouri Botanical Garden Press, St. Louis.

Calviño, A., Ashworth, L., & Moyetta, N. (2014). Fruit set increases with maleness in the andromonoecious *Acacia caven. Flora, 209,* 457-463. doi: 10.1016/j.flora.2014.06.011

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

TABLES

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

Variables	**ANOVA**			**Mean ± SD (N)**	
	df	F	p	Hermaphroditic	Staminate
Corolla length				56.35 ± 0.35 (45)	47.65 ± 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 ± 1.92 (45)	42.33 ± 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 ± 0.29 (45)	17.86 ± 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 ± 0.11 (45)	2.09 ± 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 ± 0.73 (45)	2.50 ± 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		

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 ± SD(N)**	
	df	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 ± 33% (45)	78 ± 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%

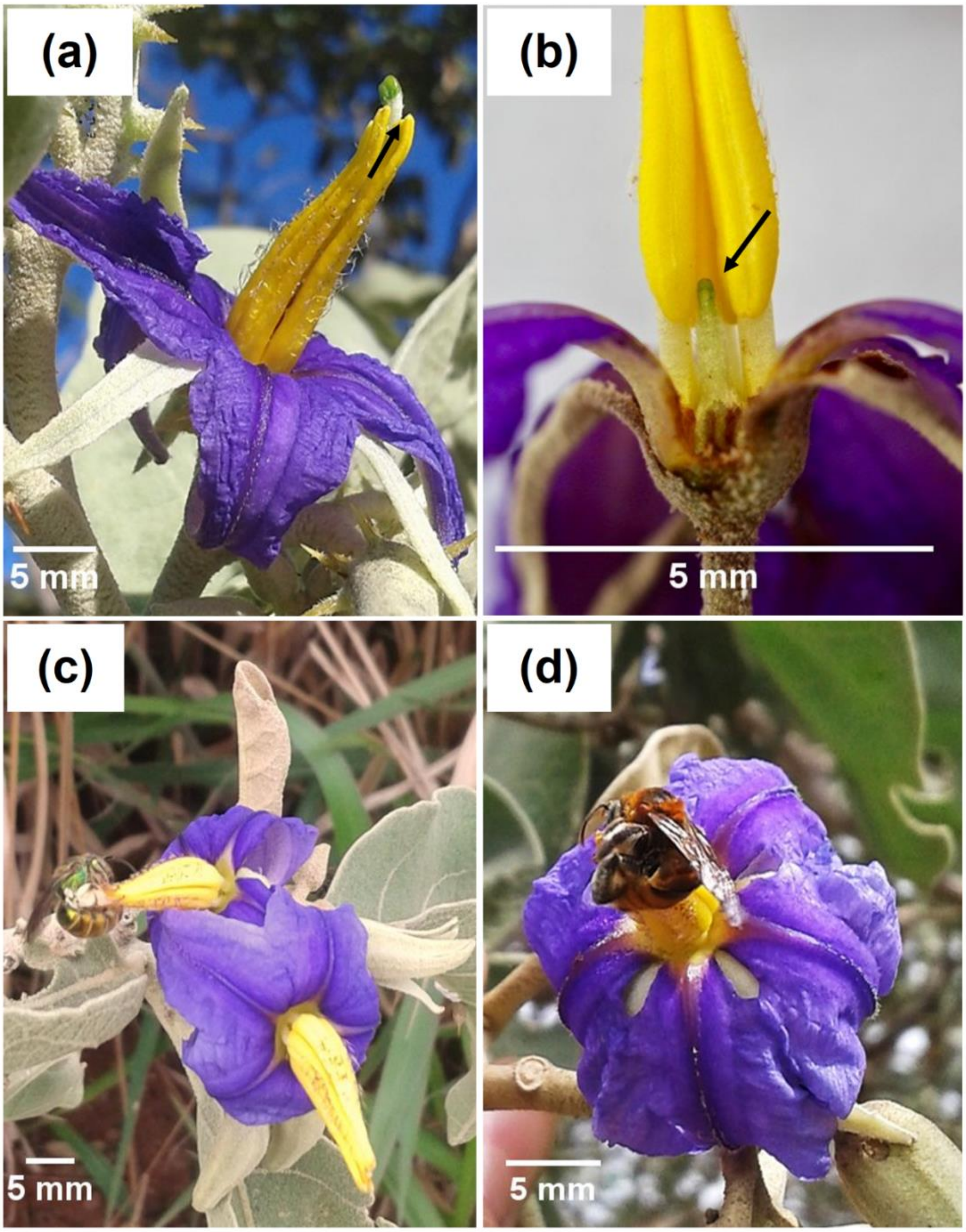
FIGURES LEGENDS

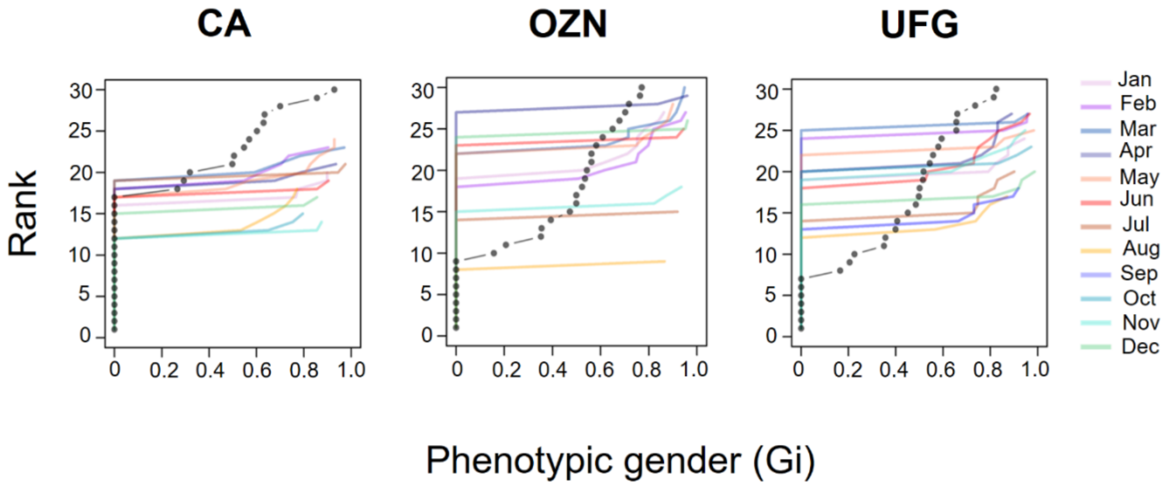
Figure 1. Flowers of *Solanum lycocarpum* A. St. -Hil. (Solanaceae) and their frequent visitors in UFG Farm, Catalão, Goiás, Brazil. (a) Hermaphroditic flower, with arrow indicating the stigma projecting above the anther cone, (b) Staminate flower, with arrow indicating the pistiloid, (c) *Exomalopis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

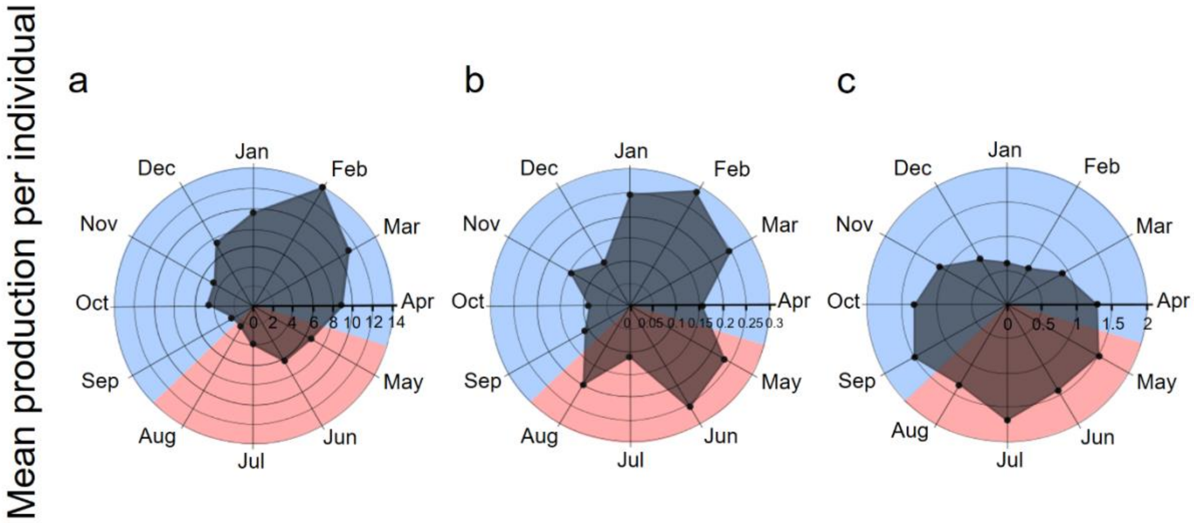
Figure 2. Variations in phenotypic gender in *Solanum lycocarpum* A. St. -Hil. (Solanaceae) from January to December 2017 in three populations in the Brazilian Cerrado. Warm colors lines represent dry months while cool colors lines represent humid months. Each line represents a month and the corresponding 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.

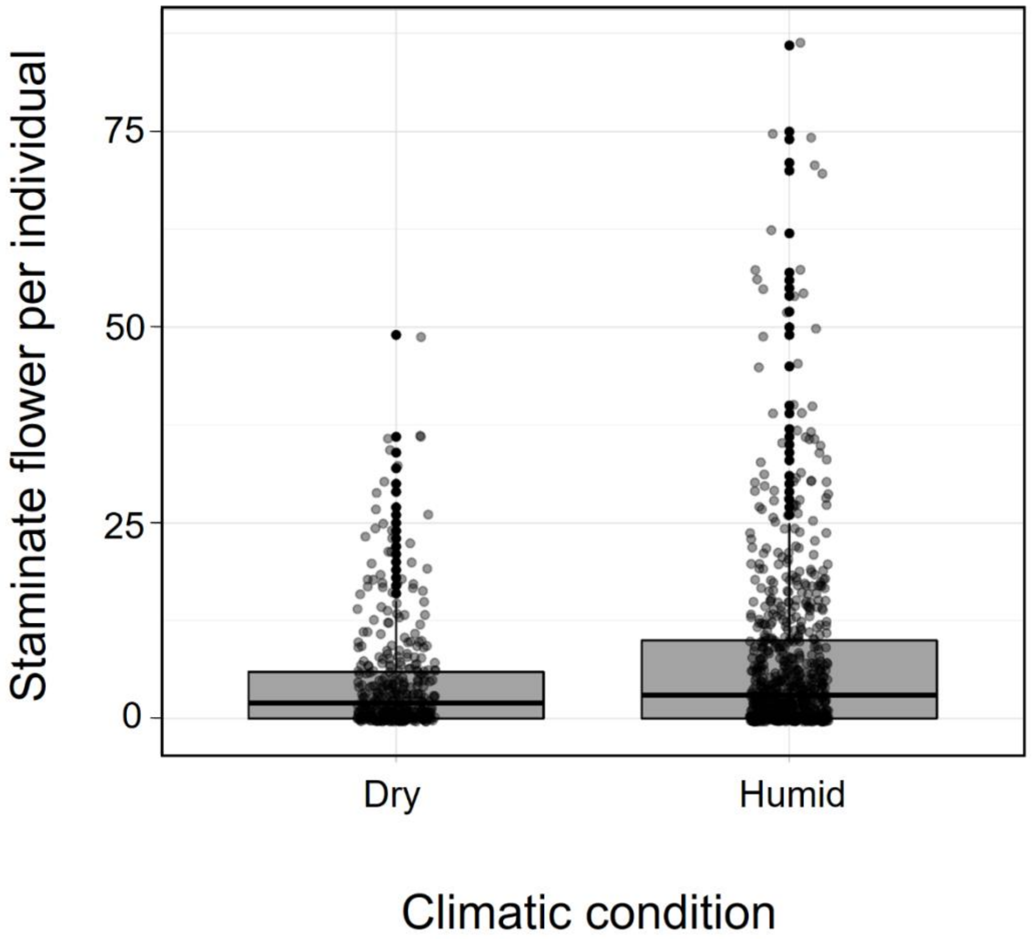
Figure 3. Mean production of (a) staminate and (b) hermaphroditic flowers, as well as (c) fruits of *Solanum lycocarpum* A. St. -Hil. (Solanaceae) per individual along the year (numeric bar) in three populations (UFG Farm, Catalão, Goiás, Brazil; Campo Alegre de Goiás, Goiás, Brazil; and Pontinhas Farm,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

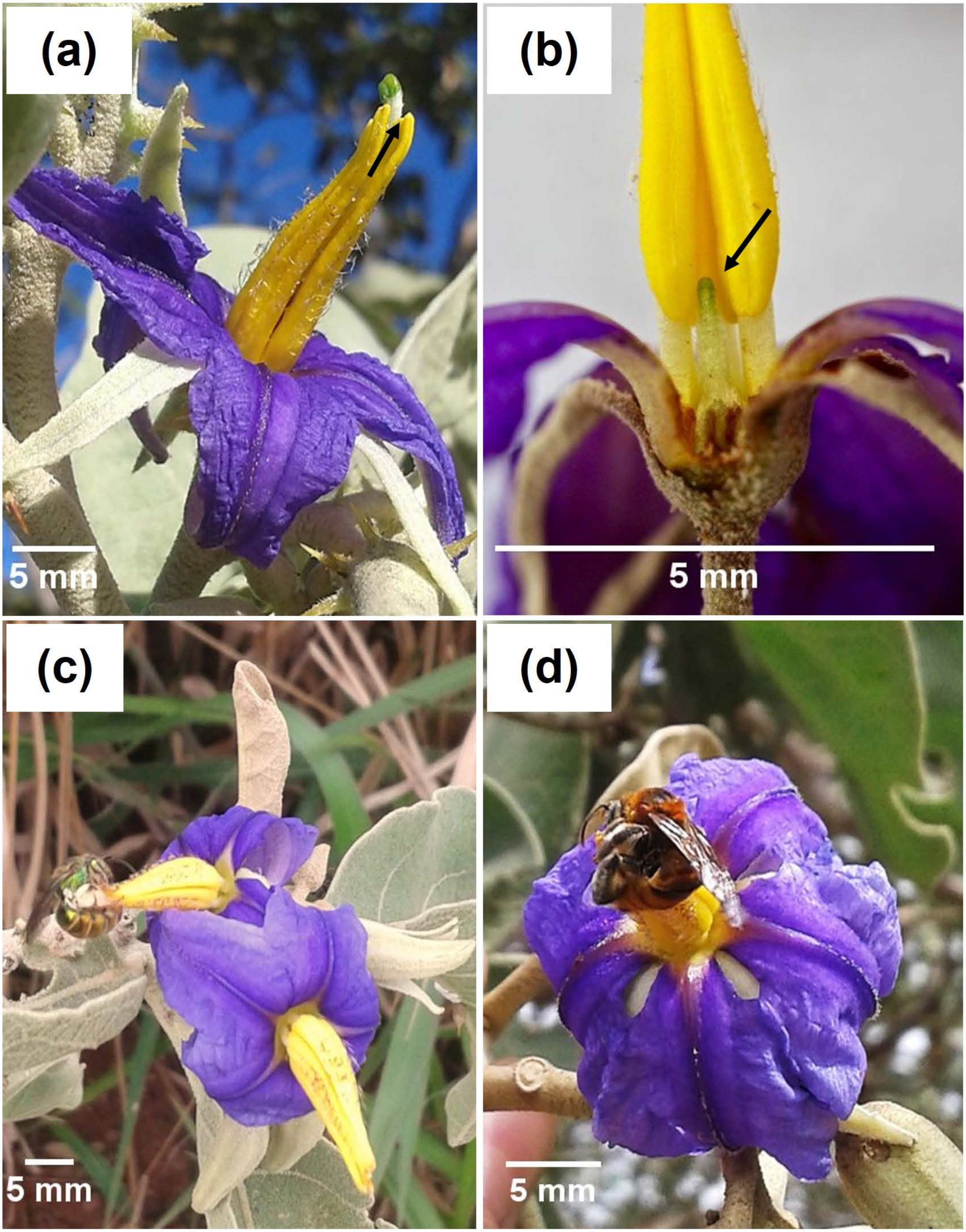
Figure 4. Number of staminate flowers produced per plant depending on the climatic condition (dry or humid) in *Solanum lycocarpum* A. St. -Hil. (Solanaceae). In the boxplots, dark lines indicate the medians, boxes represent second and third quartiles and whiskers represent first and fourth quartiles.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

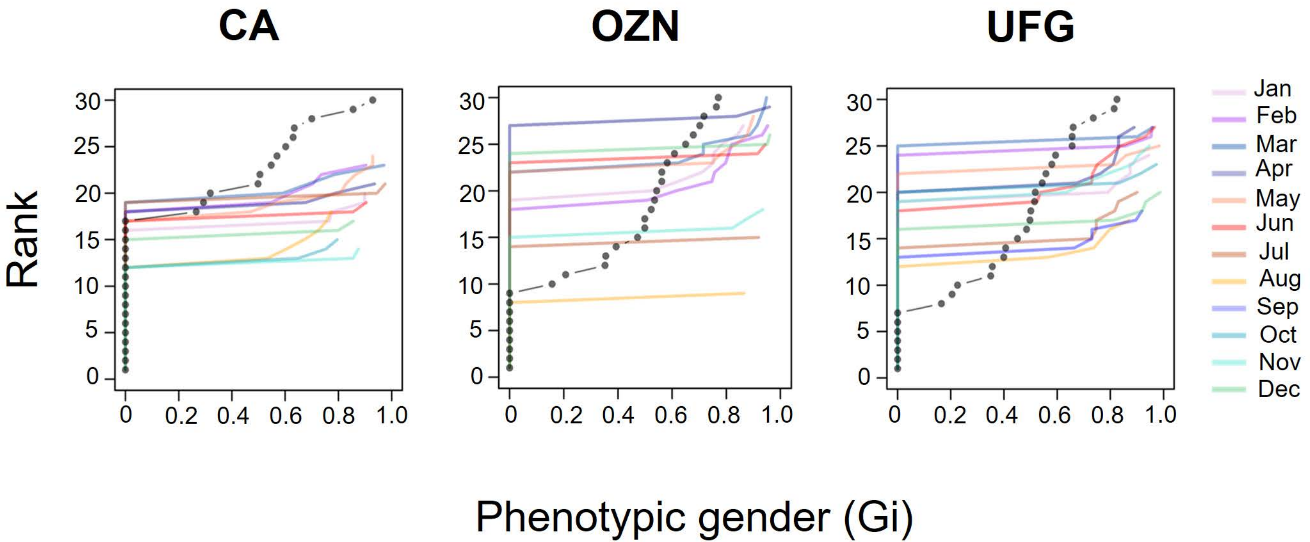

(a)
5 mm
(b)
5 mm
(c)
5 mm
(d)
5 mm

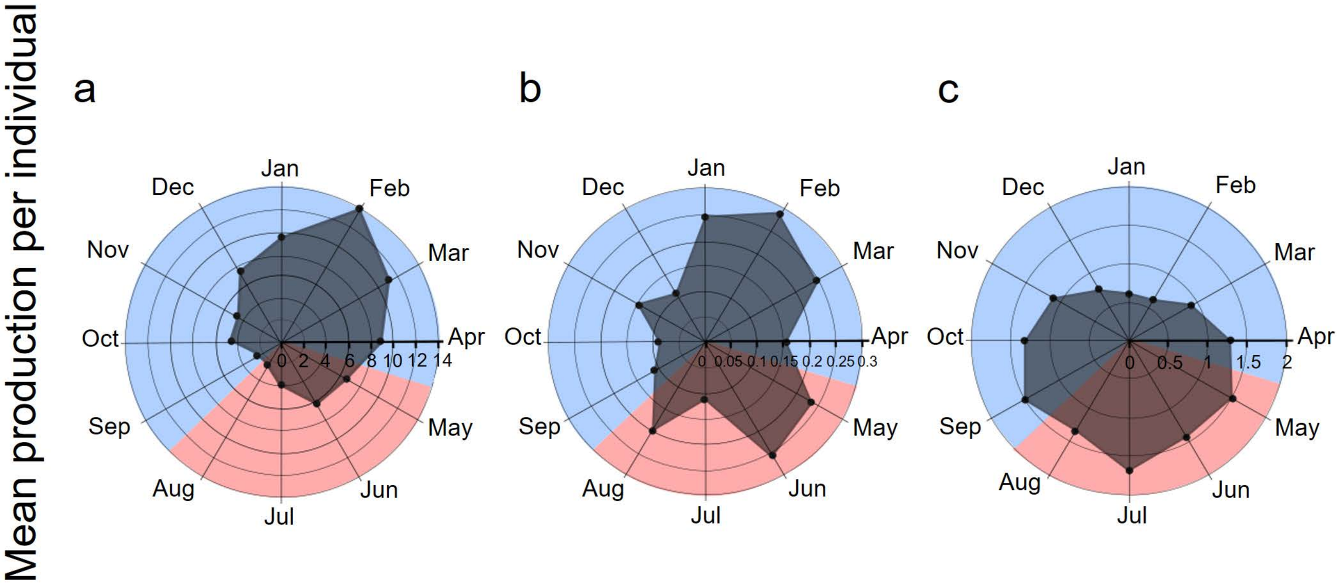

CA
OZN
UFG
Rank
30
25
20
15
10
5
0
0
0.2
0.4
0.6
0.8
1.0
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Phenotypic gender (Gi)

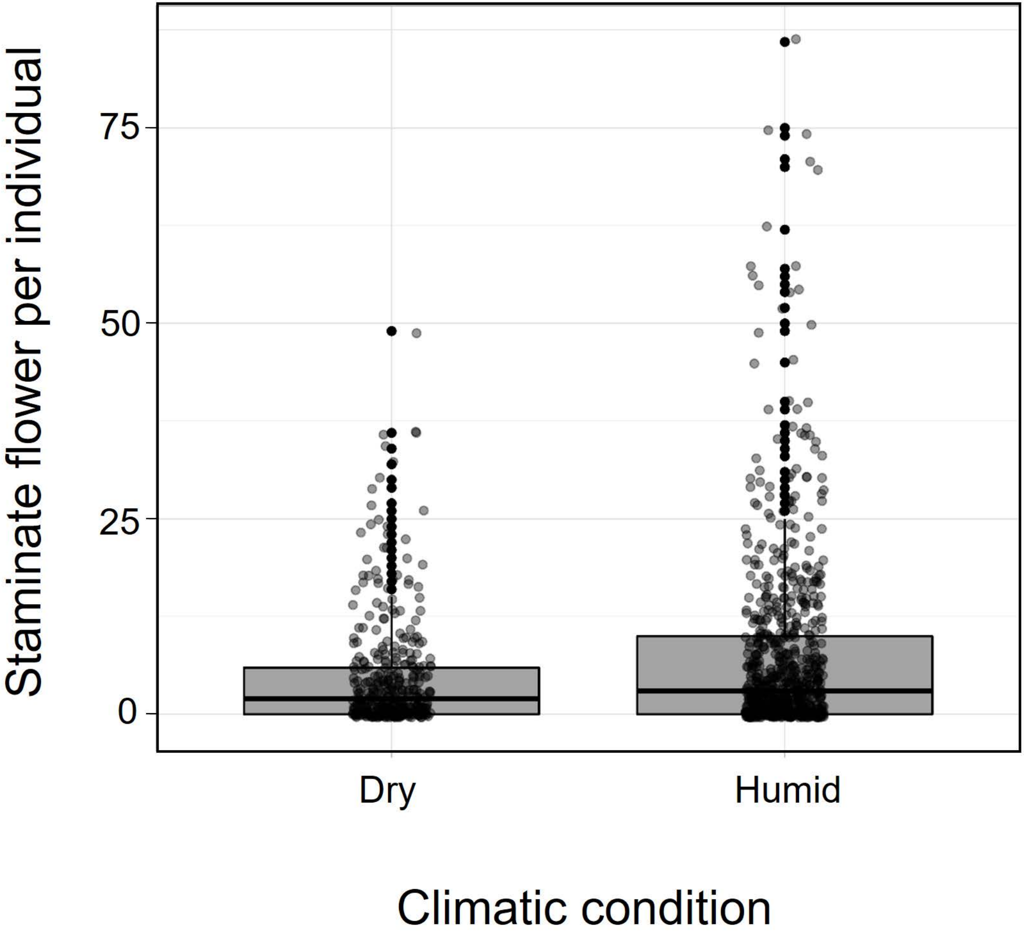

Mean production per individual
a
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
0 2 4 6 8 10 12 14
b
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
0 0.05 0.1 0.15 0.2 0.25 0.3
c
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
0 0.5 1 1.5 2


Staminate flower per individual
75
50
25
0
Dry
Humid
Climatic condition


(a)
5 mm
(b)
5 mm
(c)
5 mm
(d)
5 mm

CA
OZN
UFG
Rank
30
25
20
15
10
5
0
0
0.2
0.4
0.6
0.8
1.0
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Phenotypic gender (Gi)


Mean production per individual
a
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
0
2
4
6
8
10
12
14
b
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
0
0.05
0.1
0.15
0.2
0.25
0.3
c
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
0
0.5
1
1.5
2


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
Augochloropsis sp. 1 (Cockerell, 1897)	2.72	2.62	4.34	2.53	10.12	30 (25%)	24 (28%)	8 (13%)
Augochloropsis sp. 2 (Cockerell, 1897)	2.34	2.41	3.17	2.24	8.19			
Bombus morio (Swederus, 1787)	5.49	7.32	7.80	8.54	16.95	21 (17%)	0	1 (2%)
Bombus brevivillus (Franklin 1913)	5.22	6.23	6.46	8.83	15.67			
Bombus pauloensis (Friese, 1913)	5.01	6.56	6.88	7.19	14.80			
Centris scopipes (Friese, 1899)	10.56	12.06	12.55	13.30	29.86	3 (2%)	2 (2%)	18 (29%)
Epicharis analis (Lepeletier, 1841)	6.23	6.36	7.42	9.53	20.12	34 (28%)	6 (7%)	6 (10%)
Epicharis flava (Friese, 1900)	8.71	7.63	7.82	9.31	23.78			

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
Exomalopsis analis (Spinola, 1853)	2.32	2.37	3.08	2.84	7.38	8 (7%)	21 (25%)	7 (11%)
Exomalopsis collaris (Friese, 1899)	2.30	3.43	4.22	3.33	10.46			
Exomalopsis fulvofasciata (Smith, 1879)	3.46	4.26	5.38	4.61	11.16			
Melipona quinquefasciata (Lepeletier, 1835)	3.54	3.95	4.17	3.76	10.56	4 (3%)	2 (2%)	1 (2%)
Oxaea cf. *flavescens* (Klung, 1807)	5.59	7.24	7.61	7.51	18.00	20 (16%)	30 (35%)	10 (16%)
Paratetrapedia lugubris (Cresson, 1878)	3.76	3.90	5.84	4.11	13.34	2 (2%)	0	0
Xylocopa suspecta (Moure & Camargo, 1988)	9.27	10.38	9.50	10.13	23.26	0	0	11 (18%)
Xylocopa subcyanea (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

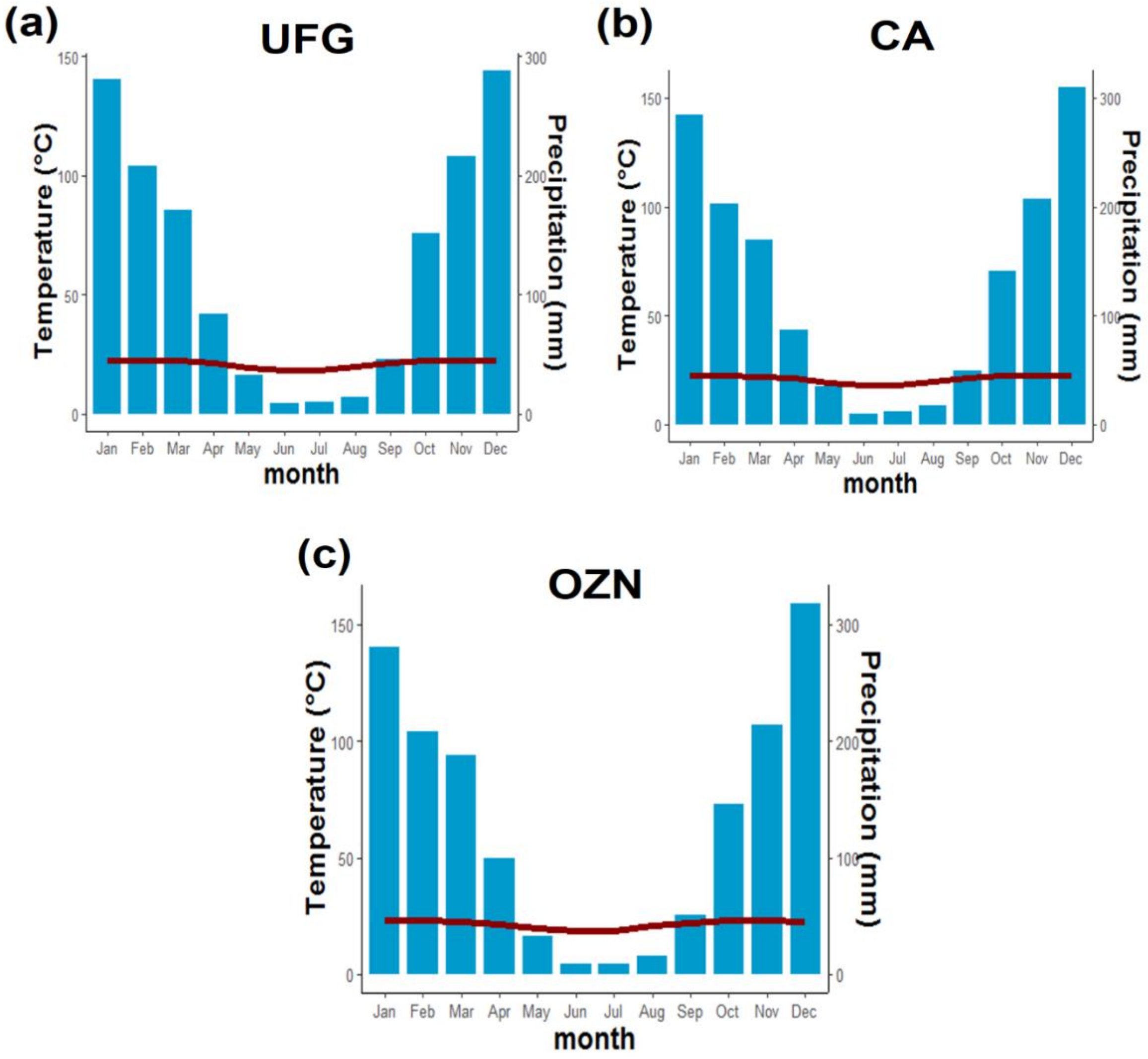


Figure S1. Classification of dry and humid months based on climate graphs. The monthly values correspond to the average temperature and precipitation between 1950-1990. In dry months, the blue bars of precipitation are below the red line of temperature, while in humid months, the bars are above the lines.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

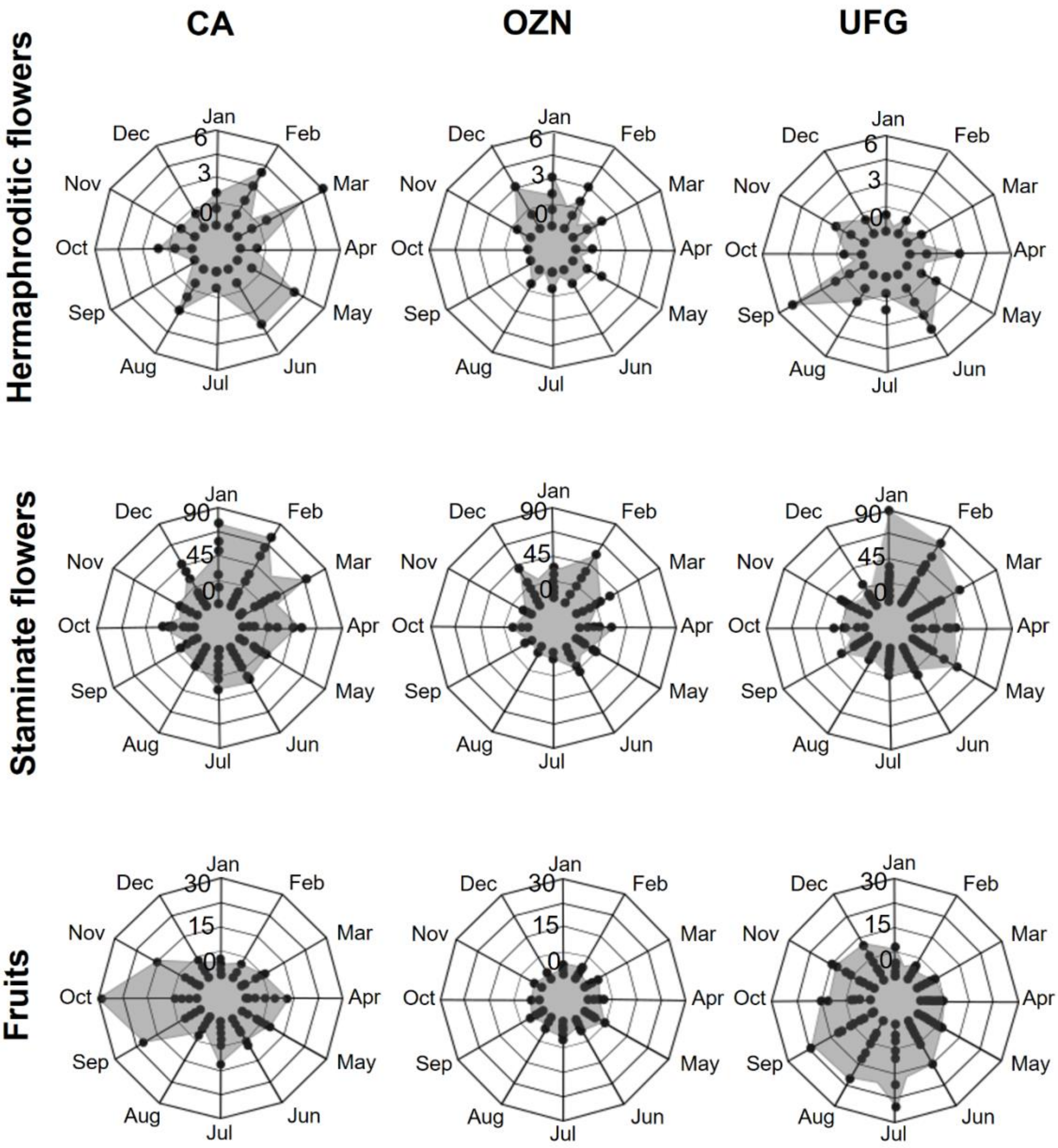
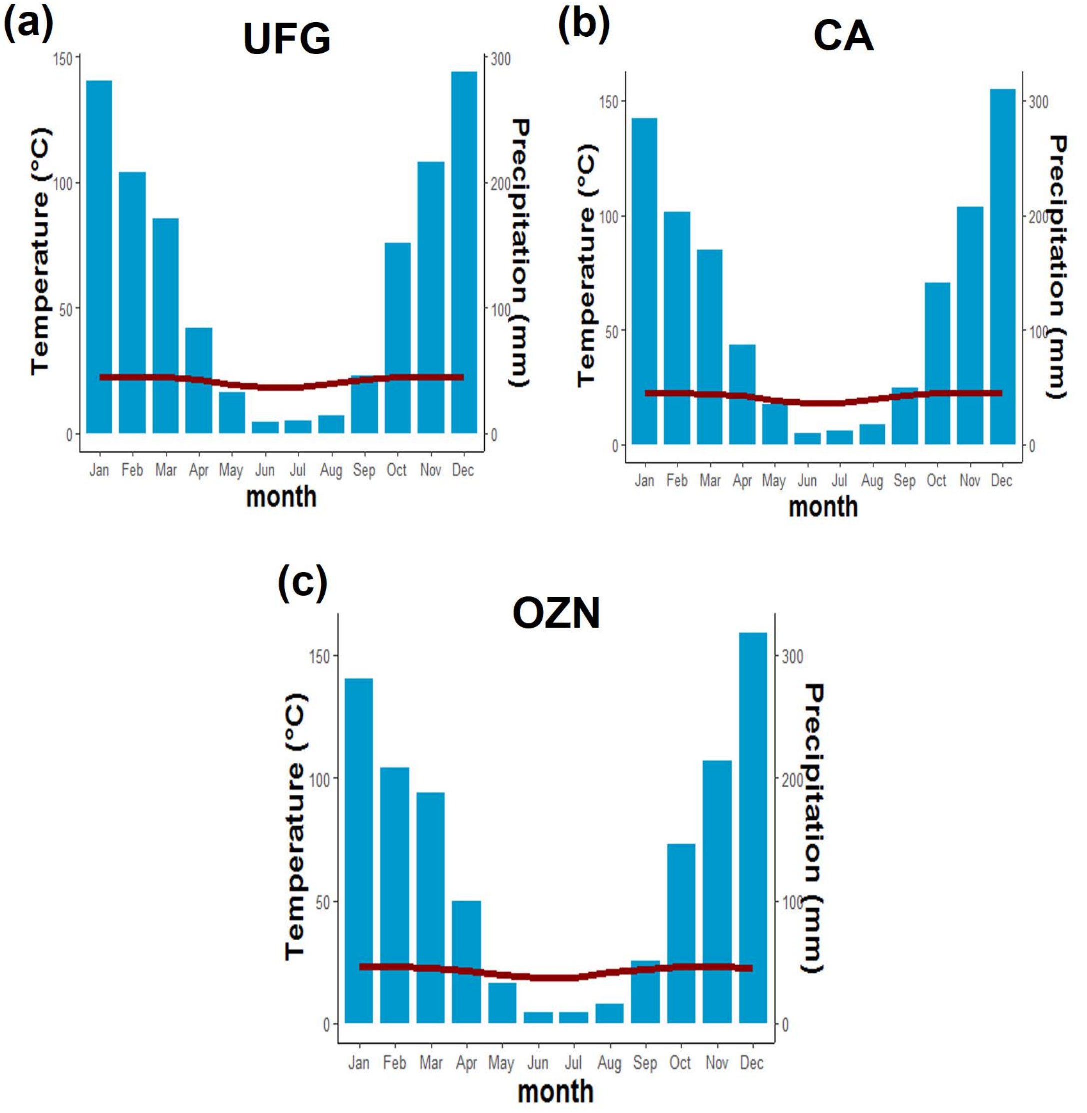
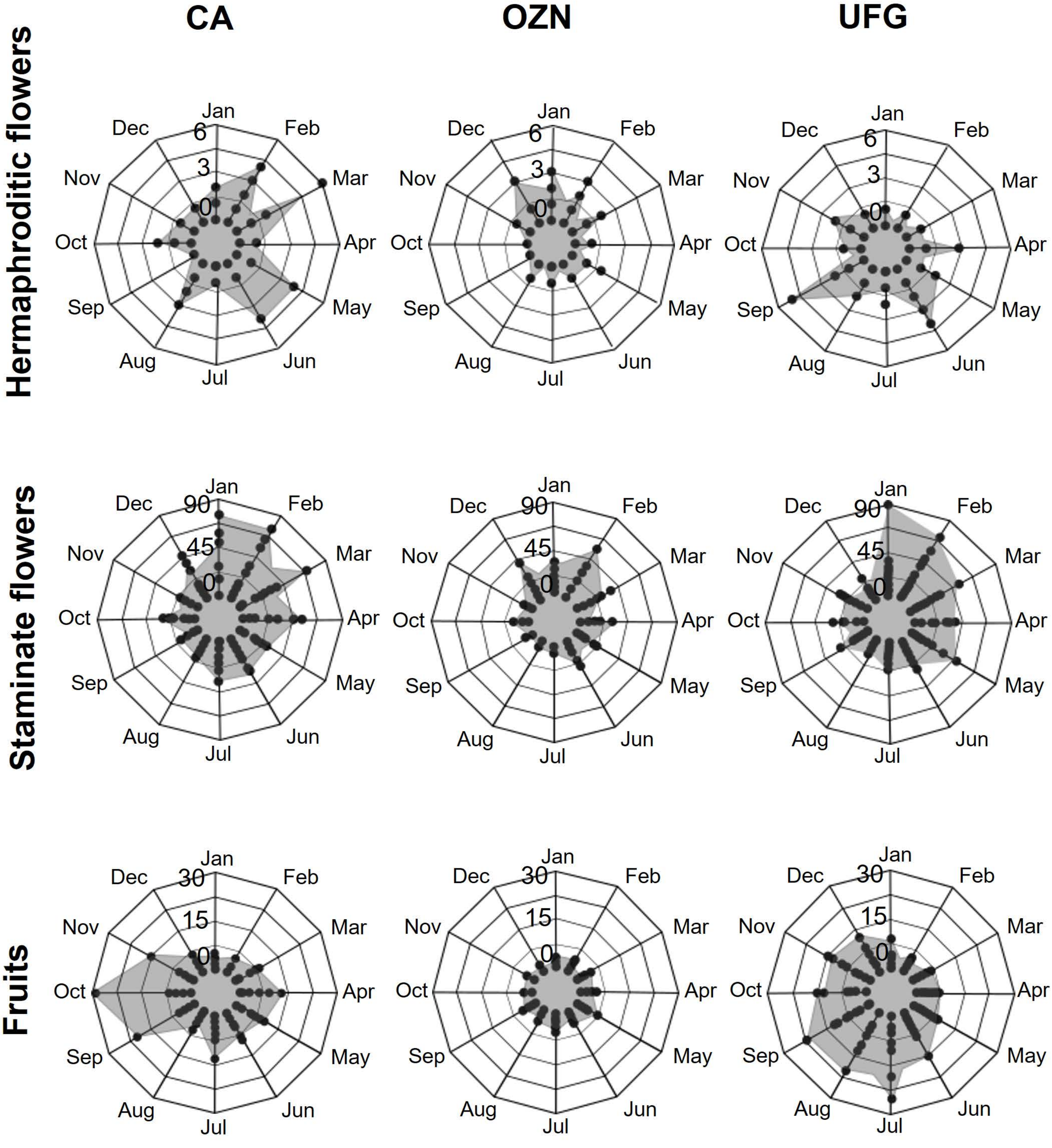


Figure S2: Production of both hermaphroditic and staminate flowers, as well as fruit production per individual of *Solanum lycocarpum* A. St. Hil. (Solanaceae) along the year (numeric bar) in each population (CA: Campo Alegre de Goiás- GO; OZN: Pontinhas Farm, Orizona- GO; UFG: UFG Farm, Catalão, GO).


(a)
UFG
Temperature (°C)
Precipitation (mm)
0
50
100
150
0
100
200
300
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
month
(b)
CA
Temperature (°C)
Precipitation (mm)
0
50
100
150
0
100
200
300
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
month
(c)
OZN
Temperature (°C)
Precipitation (mm)
0
50
100
150
0
100
200
300
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
month


CA
OZN
UFG
Hermaphroditic flowers
Staminate flowers
Fruits
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
6
3
0
90
45
0
30
15
0