

Research article

Investigating the reproductive function of floral morphs in a distylous hummingbird-pollinated plant

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Short title: The reproductive function of floral morphs in a distylous plant

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ABSTRACT

- Floral display plays an essential role in reproduction by enhancing female or male performance in flowers. The distylous *Psychotria poeppigiana* (Rubiaceae) has brightly red bracts that act as honest signals of floral resources to pollinators, with larger bracts having greater nectar availability but higher mortality, while smaller bracts produce more fruits. This raises whether the S-styled morph, characterized by larger bracts, may predominantly benefit male function, whereas the L-styled, with smaller bracts, favors female function.
- We evaluated morphological trait differences between L- and S-styled morphs, including bract size and floral traits (length, diameter, and number of flowers per bract). We then tested whether bract size influences bract mortality. We also evaluated the effect of bract size on male and female pollination performance by analyzing pollen remaining in anthers (as a proxy for pollen removal) and pollen deposition on stigmas, and assessing female reproductive success through fruit set and seed viability.
- The S-styled presented larger bracts (10.1%), and longer flowers (18.3%) than L-styled morph. Bract size did not affect pollen deposition; however, L-styled morph received about 3.3 times more pollen than S-styled. Bract size showed negative relationships with fruit set and fruit size, and a positive relationship with bract mortality. Seed traits were not influenced by bract size or morph type.
- Despite morphological differences, no marked differences in reproductive function were detected. Larger bracts may require resource costs that limit reproductive success, whereas smaller bracts may allow more efficient allocation to fruit production, increasing fruit set.

RESUMO

O display floral desempenha um papel essencial na reprodução por potencializar a performance feminina e masculina nas flores. A espécie distílica *Psychotria poeppigiana* (Rubiaceae) apresenta inflorescências envolvidas por brácteas avermelhadas que agem como sinal honesto de recursos florais para polinizadores, em que brácteas maiores possuem maior disponibilidade de néctar, mas uma taxa de mortalidade maior, e brácteas menores produzem mais frutos. Esses padrões indicam que os morfos florais podem estar desempenhando funções reprodutivas distintas, sendo o morfo brevistilo, com brácteas maiores, potencializando a função masculina (remoção de pólen), enquanto o morfo longistilo, com brácteas menores, maximizando a função feminina. Comparamos entre os morfos os atributos morfológicos, como o tamanho da bráctea, número de flores, tamanho das flores e mortalidade das brácteas. Também avaliamos os efeitos do tamanho das brácteas na performance de polinização feminina e masculina, através da análise de pólen restante nas anteras e deposição de pólen nos estigmas, além de acessar o sucesso reprodutivo feminino através da formação de frutos e viabilidade das sementes. O morfo brevistilo apresentou brácteas e flores maiores quando comparado ao morfo longistilo. O tamanho da bráctea não afetou a deposição de pólen, porém, o morfo longistilo recebeu cerca de 3.3 vezes mais pólen que o morfo brevistilo. Foi encontrado uma relação negativa entre tamanho da bráctea e frutificação, bem como entre o tamanho da bráctea e tamanho de fruto. Além disso, o tamanho de bráctea foi relacionado positivamente com a mortalidade. Os atributos das sementes não foram influenciados pelo tamanho de bráctea ou pelo morfo floral. Apesar de diferenças morfológicas, não foram encontradas diferenças marcantes na função reprodutiva entre os morfos. O alto custo de manutenção de brácteas maiores pode estar criando um trade-off que limita o seu sucesso reprodutivo. Por outro lado, um menor investimento no tamanho das brácteas pelas brácteas menores, pode permitir uma alocação de recurso mais eficiente para a formação de frutos.

Palavras-chave: biologia reprodutiva, distília, display floral, função reprodutiva, polinização, sistemas reprodutivos.

INTRODUCTION

Pollination is one of the main drivers of floral trait diversification in animal-pollinated plants (Willmer 2011; van der Niet & Johnson 2012). The angiosperms have evolved diverse floral adaptations, such as colors (Lunau & Maier 1995; Van Der Kooi *et al.* 2019), scents (Wright & Schiestl 2009), floral symmetry (Citerne *et al.* 2010), and floral display size (Thompson 2001), that influence pollinator attraction. Variation in such traits can influence pollinator visitation patterns, behavior, and effectiveness, ultimately influencing plant reproductive performance, including pollen production and transfer, as well as fruit and seed formation (Waser 1983; Ohashi & Yahara 1999).

Variation in floral display at the intra-population level, a key reproductive trait to promote cross-pollination (Harder & Barrett 1995), is frequently associated with asymmetric contributions to fitness in male and female functions (Karron & Mitchell 2012). Larger floral displays increase pollinator attraction, thereby enhancing pollen transfer and male function, a pattern that is widely reported (Goulson 1999; Ohashi & Yahara 1999; Armbruster *et al.* 2005; Glaettli & Barrett 2008). Moreover, the increase in pollen production in many angiosperm species is linked to an increase in floral display size (Da Cunha & Aizen 2023). By contrast, in *Mimulus ringens* (Phrymaceae), larger floral displays enhance fruit set, whereas smaller displays promote higher siring success (Karron & Mitchell 2012).

Flowers may differ in their functional sexual roles, acting predominantly as pollen donors (Da Cunha & Aizen 2023) or pollen receivers. This functional divergence has long been recognized in polymorphic systems, especially in heterostylous species, in which floral morphs commonly exhibit sexual specialization, resulting in distinct male and female reproductive success (Darwin 1887; Charlesworth 1989; Barrett 1992; Ornelas *et al.* 2004; Valois-Cuesta *et al.* 2012; Pannel 2018). For example, in distylous *Linum perene* (Linaceae), the L-styled produced less pollen but matured more seeds compared to the S-styled (Nicholls 1986). By contrast, in *Psychotria rubra* (Rubiaceae), the S-styled produces pollen grains but does not set fruit, whereas the L-styled produces no pollen and set fruits (Watanabe *et al.* 2014). In *Chassalia corallioides* (Rubiaceae), the S-styled morph also does not set fruit, whereas the L-styled morph produces fruit (Pailler *et al.* 1998). These examples highlight that when female and male functions vary between morphs, distylous systems may function as functional dioecy (Barrett 2019).

A similar pattern may also occur for *Psychotria poeppigiana* Müll. Arg. (Rubiaceae) characterized by reddish bracts that serve as honest signals to pollinators, with larger bracts and flowers containing more nectar (Trevizan *et al.* 2025). Despite the increased allocation of nectar, which might imply greater pollinator attraction and potentially higher fruiting rates in larger bracts, smaller bracts actually have a higher fruiting rate, whereas larger bracts lead to increased inflorescence mortality, indicated by a higher rate of spontaneous bract wilting and fall (Trevizan *et al.* 2025). Furthermore, this species exhibits distyly, characterized by individuals with two distinct floral morphs: L-styled and S-styled (Coelho & Barbosa 2004). S-styled individuals are characterized by larger bracts and flowers, as well as a greater number of flowers per bract compared to L-styled morphs (Trevizan *et al.* 2025). These morphological differences raise the question of whether the two morphs contribute differently to reproductive function within the population, potentially partitioning functions related to male and female performance according to bract size variation. However, the specific reproductive functions associated with each floral morph and how bract size may mediate these roles remain unknown.

Here, we aimed to investigate the reproductive function of the floral morphs of *P. poeppigiana*. We addressed the following questions: (1) Are there differences between morphs regarding bract and flower traits? (2) Do bract size influences female and male pollination performance between S- and L-styled morphs? (3) Do bract size influences the rate of fruit-set, seed set, and offspring quality across morphs? We hypothesized that the S-styled morph, characterized mainly by larger bracts, predominantly enhances male function, while the L-styled morph, with predominantly smaller bracts, favors female function. Specifically, we predicted the S-morph would be positively associated with pollen export, whereas the L-morph would be positively related to pollen deposition and fruit set. Based on this, we can achieve a better understanding of the sexual function of *P. poeppigiana* floral morphs.

MATERIAL AND METHODS

Study area and study species

The study was carried out from October to February 2024/2025 in the Itororó Park Club (CCPIU), Uberlândia, Minas Gerais state, southeast Brazil (127 ha; 18°55'S; 48°17'W). The studied population is located in the Cerrado biome, the Brazilian savanna, specifically within a riparian forest. The region is in Aw climate, a tropical climate with dry winters, according to Köppen Climate classification (Martins *et al.* 2018).

P. poeppigiana is a native subshrub (Flora e Funga do Brasil 2026) characterized by small, yellow, sessile flowers with tubular corollas that are odorless and produce nectar as the main resource for its pollinators, which are hummingbirds (Coelho e Barbosa 2004; Trevizan *et al.* 2025). The flowers are arranged in terminal capitate inflorescences, with pentamerous one-day, epigynous, bi-ovulate flowers, encircled by two brightly red bracts (Coelho & Barbosa 2004). The flowering period is between June and January, and the fruiting period between October and March (Valois-Cuesta 2009), when the blueish fruits ripen. The species exhibits distyly, with thrum and pin floral morphs, and shows self- and intramorphic incompatibility system (Coelho & Barbosa 2004).

Bract traits: size and mortality

We tagged 110 bracts (68 S-morph and 50 L-morph) from 18 individuals using masking tape with information written in pencil. During fieldwork, standardized photographs of the bracts were taken by placing them on graph paper and positioning them perpendicular to the camera's focal plane (Canon Ti3 Eos camera; Ef-s 18-55mm lens). Bract sizes were analyzed using geometric morphometry method (GMM) to measure 2D morphological variation (Bookstein 1991; Zelditch *et al.* 2012). The images resulting from this process were analyzed using the TPSUtil (Version 1.80; Rohlf 2015) and tpsDig program (Version 2.31; Rohlf 2015; Rohlf & Slice 1990; Zelditch *et al.* 2012). We performed the geometric morphometric analyses with the *geomorph* package in software R (Version 2025.05.1; see details in Trevizan *et al.* 2025).

The selected bracts were monitored throughout the study period to record mortality. Bract mortality was determined by the presence of fallen bracts on the ground or completely brown wilting bracts still attached to the branches. Those inflorescences with fallen or wilting bracts never formed fruits.

Flower traits: length, diameter and number

In the selected bracts inflorescences, the total number of flowers was quantified by counting both open flowers and floral scars (as in Trevizan *et al.* 2025). Between 2–3 flowers per bract were collected in the late morning (between 0900 and 1100 h) and measured for flower length and diameter using a digital caliper (N = 55 to S-styled and N = 44 to L-styled). We

defined flower length as the distance from the base of the corolla to the opening of the corolla tube, and the corolla opening as the diameter of the flower.

Pollination performance

i) Pollen deposition on stigmas

The collected flowers (N = 55 S-styled and N = 44 L-styled) were dissected, and the pistils were removed and preserved in FAA (50%) in 1.5 mL Eppendorf tubes. The pistils were then softened in 10N NaOH at 60°C for 15 minutes, thoroughly rinsed in tap water, and stained with aniline blue (adapted from Martin 1959). Under a fluorescence microscope the pollen grains on the stigma were observed and counted.

ii) Pollen remaining in anthers

The stamens were collected from the dissected flowers and preserved in 100 µl of ethanol (70%) in 1.5 ml Eppendorf tubes (N = 55 S-styled and N = 44 L-styled). We also collected 1-2 floral buds that were preserved using the same procedure (N = 41 S-styled and N = 24 L-styled). Stamens for each collected flower were mashed using a glass rod and transferred to a solution containing 150 µl of ethanol (70%) and 50 µl of glycerin (3:1). Using a vortex, the solution was stirred for 60 s, and a 10µl sample was transferred to a Neubauer chamber (hemocytometer) for pollen grain estimation, considering the total solution and chamber volumes (Trevizan *et al.* 2023). Floral buds were processed using the same pollen-counting methodology to quantify pollen production.

iii) Fruit set

We considered effective fruiting, meaning the full development of fruits and seeds, as our measure of reproductive success for the selected bracts inflorescences. When the fruits were fully ripe (mid-December), all intact mature fruits were collected (N = 17 S-styled; N = 7 L-styled), and their weights and sizes were measured. Weight was measured using a precision scale, while size was determined through standardized photographs (Canon Ti3 Eos camera; EF-S 18-55mm lens), on which the fruits were placed on a piece of graph paper in a

perpendicular orientation to the focal plane of the camera lens. The images resulting from this process were analyzed using ImageJ (Version IJ 1.46r; Schneider *et al.* 2012).

iv) Seed production and offspring quality

To assess seed traits, we quantified, measured, and tested the viability of seeds obtained from the collected fruits. Seed weight was measured using a precision scale (N = 34 S-styled; N = 10 L-styled). Then, seed size was determined from standardized photographs (Canon Ti3 Eos camera; Ef-s 18-55mm lens), in which seeds were placed on graph paper, oriented perpendicularly to the camera's focal plane (N = 34 S-styled; N = 10 L-styled). The resulting images were analyzed using ImageJ (Version IJ 1.46r; Schneider *et al.* 2012). Finally, seed viability was evaluated by visual inspection, germination, and tetrazolium tests (França-Neto & Krzyzanowski 2022). Moldy and soft seeds were classified as non-viable. The remaining seeds were disinfected in commercial sodium hypochlorite (10%), rinsed in distilled water, and placed in paper trays (2.4 cm diameter) inside Gerbox containers sterilized with ethanol (70%) and UV light for 24 h. Trays were maintained at 25 °C under continuous white fluorescent light (9W/6500K), and seeds moistened with distilled water and monitored every three days for 60 days for radicle protrusion. Non-germinated seeds were then subjected to the tetrazolium test (1.0%), longitudinally sectioned, stained for 3 h in the dark at 25 °C, and classified as non-viable when showing no staining and/or flaccid embryo and/or dark purple color.

Statistical analysis

We first explored the differences between morphs regarding bract and floral traits. Specifically, we considered bract size, as well as flower length, flower diameter, and total number of flowers per bract, to confirm distinctions between L- and S-styled floral morphs. We adjusted separate models for each response variable, using floral morph as a predictor variable and plant individual as the random effect. We applied a generalized linear mixed model (GLMM) with *Gamma* distribution and *log* link function (continuous/positive data), except for the number of flowers response variable, where we used *binomial negative 2* distribution and *log* link function (count data) (Dunn & Smyth 2018).

After assessing bract and floral traits differences between morphs, we evaluated the effect of bract size on aspects of pollination performance. Specifically, we focused on pollen deposition on stigmas as a measure of female performance, and remaining pollen in the anthers as a proxy of pollen removal and a measure of male performance. We constructed separate models for each of these response variables. To analyze whether bract size was related to pollen deposition on stigmas, we applied a GLMMs with a negative *binomial 2* distribution and log link function (for continuous/overdispersed count data; Dunn & Smyth 2018). To analyze whether bract size was related to remaining pollen in the anthers, we used a GLMM with a *negative binomial 1* distribution (count data; Dunn & Smyth 2018). We considered bract size and morph type as predictor variables, while branch nested within individual was considered a random effect. We also tested the difference of pollen production between morph individuals by applying for a T test.

To investigate whether reproductive success (i.e. fruit set) was affected by bract size, we applied a GLMM with *binomial* distribution and *logit link* function. To create our proportional response variable, we took the number of unformed fruits (i.e. number of failures) and the number of formed fruits (i.e. number of successes), and combined these two variables through the *cbind* function in R-base package (Crawley 2013). We considered bract size and morph as predictor variables and individual as random effect.

Additionally, we investigated whether fruit traits were affected by bract size. For this, we applied two GLMM with *Gaussian* distribution and *identity* link function (continuous data; Dunn & Smyth 2018). We considered bract size and morph type as predictor variables, while branch nested within individual was the random effect. The response variables were fruit area (mm²) and fruit perimeter (mm). We also examined the effect of bract size on seed traits with a similar GLMM framework (*Gaussian* distribution, *identity* link function). In this case, bract size and morph type were used as predictor variables, and fruit nested within branch, and branch nested within individual were specified as random effects. The response variables were seed area (mm²), seed perimeter (mm), and seed weight (g). For seed weight, however, no random effect was included due to insufficient data leading to non-estimable effects. We also investigated the effect of bract size on seed germination. For this, we applied a GLMM with *binomial* distribution and *logit link* function. We considered bract size and morph type as predictor variables, while individual was the random effect. The response variable was the presence/absence of seed germination.

Finally, we investigated whether bract mortality was affected by bract size. We applied a GLMM with *binomial* distribution and *logit* link function. Bract mortality was used as the response variable, while bract size and morph type were included as predictor variables.

We used the glmmTMB R-package to adjust all models (version 1.1.11; Brooks et al. 2017). We checked model adjustment using the QQ plot of residuals and the plot of predicted vs. residuals values for each model, by simulating residuals 250 times in the DHARMa R-package (version 0.4.7; Hartig & Lohse 2022). To test the significance of the models, we applied a type II Wald Chi-square test, except for the binomial model testing mortality (presence/absence), for which we applied a Likelihood Ratio (LR) test, using the car R-package (version 3.1.3; Fox *et al.* 2018). To report and plot results, we used model-adjusted values that were back-transformed using the *ggpredict* function from the *ggeffects* R-package (version 2.2.1; Lüdtke 2018). All analyses were performed in R software (R Core Team 2025).

RESULTS

Bracts and floral traits

We confirmed differences in bract and flower traits between morphs. The bract size of the S-styled morph was 10.1% larger than that of the L-styled morph ($\chi^2 = 3.831$, $df = 1$, $p = 0.05$; Fig. 1A). Regarding floral traits, flower length of the S-styled morph was 18.3% larger than that of the L-styled ($\chi^2 = 28.608$, $df = 1$, $p < 0.001$; Fig. 1B). There was no evidence for difference between L- and S-styled morphs in flower diameter ($\chi^2 = 0.736$, $df = 1$, $p = 0.390$). Although the S-styled morph had 43.21% more flowers than the L-styled morph, this difference was not statistically significant ($\chi^2 = 2.096$, $df = 1$, $p = 0.147$). Bract size had a positive effect on bract mortality, with bract mortality increasing with the size of bracts ($\chi^2 = 6.833$, $df = 1$, $P = 0.008$; Fig. 4). We found no effect for morph type on bract mortality ($\chi^2 = 2.768$, $df = 1$, $P = 0.09$).

Pollination performance

We found significant effects for pollen deposition on stigmas between morphs ($\chi^2 = 8.085$, $df = 1$, $p = 0.004$; Fig. 2A). L-styled stigmas received about 3.3 more pollen than S-styled stigmas. In contrast, bract size had no significant effect on pollen deposition ($\chi^2 = 0.021$, $df = 1$, $p = 0.884$). Regarding the amount of pollen remaining in anthers (pollen removal), neither bract

size nor morph type had a significant effect ($\chi^2 = 3.506$, $df = 1$, $p = 0.061$ and $\chi^2 = 0.276$, $df = 1$, $p = 0.599$, respectively). We found no significant differences in pollen production between L- and S-styled morphs ($t = -0.37$, $df = 42.63$, $p = 0.71$).

Female reproductive success

Bract size had a negative effect on fruit-set ($\chi^2 = 4.860$, $df = 1$, $p = 0.027$; Fig. 3A). Morph type did not significantly affect fruit-set proportion ($\chi^2 = 0.779$, $df = 1$, $p = 0.377$). Bract size and morph showed an interactive effect in fruit area (Fig. 3B, Table S1; Fig. 1C, Table S1). S-styled flowers produced fruits with a 73.1% larger area than those of L-styled flowers (Fig. 1C). For fruit perimeter, neither bract size nor morph type had a significant effect (Table S1). Finally, we found no significant effects of bract size or morph on seed area, seed perimeter, seed weight, or seed germination (Table S1).

DISCUSSION

We found bracts differences between S-styled and L-styled morphs, with S-styled morphs exhibiting a larger floral display (bracts and corolla), but no significant difference in the number of flowers, corroborating Trevizan *et al.* (2025). Our results also confirmed the negative relation between bract size and the number of fruits produced (fruit-set), and bract/inflorescence mortality previously described (Trevizan *et al.* 2025). However, although pollen deposition on stigmas was not affected by bract size, L-styled flowers showed higher pollen deposition. The amount of pollen remaining in anthers showed no significant effect from either bract or morph type. We also did not find a difference in pollen production between morphs. Finally, although smaller bracts and S-styled flowers produced larger fruits (area), seed traits were not influenced either by bract size nor morph type. These general trends and consequences are discussed below.

We predicted that the S-styled morph, associated with larger display size (larger bracts) would function primarily as a pollen donor, maximizing pollen export, whereas the L-styled, associated with smaller bracts, would function mainly as a pollen recipient, enhancing pollen deposition. While previous studies have demonstrated that larger floral displays can improve male reproductive functions by boosting pollinator visitation (Pickering & Arthur 2003), promoting increased pollen production (Da Cunha & Aizen 2023), and enhancing both pollen

transfer and male reproductive success (Arista & Ortiz 2007), our findings contradict this expectation, as neither the larger bract display of *P. poeppigiana* enhanced male performance nor the smaller bracts favored female performance. However, we found a morph effect, with pollen deposition higher in L-styled flowers, which may reflect adaptations associated with distyly polymorphism itself. The L-styled morph, due to approach herkogamy, has its stigma positioned above the anthers and exposed outside the floral tube facilitating contact with the pollinator's body, which enhances pollen reception (Nishihira *et al.* 2000; Cardoso *et al.* 2018), and may increase the likelihood of capturing inter-morph (i.e. legitimate) pollen (Brys *et al.* 2008). Conversely, in the S-styled morph, the stigma is positioned below the anthers and inside the corolla tube. This positioning may reduce the deposition of legitimate pollen grains due to increased contact with the tube walls, resulting in lower pollen deposition rates and a greater likelihood of receiving illegitimate pollen (Trevizan *et al.* 2024). In contrast, the higher pollen deposition observed in the L-styled stigmas may be explained by the more efficient export of disassortative pollen transfer from S-styled anthers to L-styled stigma, a pattern also reported for *Primula vulgaris* (Keller *et al.* 2014) and other distylous species (Trevizan *et al.* 2024). Nevertheless, pollen remaining in anthers, did not differ between morphs nor among bract sizes. If there is no difference in pollen removal from anthers between L- and S-styled morphs, our results suggest similar visitation rates and removal effectiveness between bracts and morphs. Such results contradict our initial prediction and suggest male function is not really favoured in S-styled larger-bracted flowers.

We confirmed the negative relationship between bract size and fruit set, whereas a positive relationship between bract size and bract mortality, consistent with previous studies (Trevizan *et al.* 2024). We expected a trade-off between attraction and reproduction, in which larger bracts invest in pollinator attraction at the expense of reproductive success, whereas smaller bracts allocate more resources to fruit and seed production. Consistent with this expectation, larger bracts showed lower fruit set and higher mortality rates, highlighting the trade-off between attraction and reproduction. Floral resources can be allocated among different structures and functions (Schoen & Ashman 1995), and the high energetic costs associated with attractiveness may limit investment in fruit production (Lloyd & Barrett 1996; Sargent *et al.* 2007; Teixido & Valladares 2013). The combination of larger bracts, a higher number of flowers, and increased flower size indicates a strong investment in attractiveness, which likely explains both the reduced fruitification and the higher mortality due to maintenance costs in larger bracts. Nevertheless, it is known that a larger floral display directly enhances pollinator

visitation (Sargent *et al.* 2007), and consequently pollen removal and transfer, suggesting that investment in large bracts may represent a necessary risk to ensure pollination. Thereby, smaller bracts invest in offspring rather than attraction, presenting higher fruit set and lower mortality rates.

We also found a negative relationship between bract size and fruit area, regardless of morph, supporting our hypothesis that smaller bracts allocate resources to fruit production. Since both fruit production (Obeso 2002) and bract maintenance (Song *et al.* 2024) are energetically costly, reduced investment in bract size may be compensated by increased allocation in fruit area, characterizing a trade-off between these structures. Moreover, larger fruits may enhance seed dispersal (Sargent *et al.* 2007), reinforcing that greater resource allocation to fruits represents a higher investment in reproductive output. One interesting finding was that the S-styled produced larger fruits compared to the L-styled morph, possibly reflecting allometric pattern (Andersson 1996; Weiner 2004).

CONCLUSION

We confirmed morphological differences between morphs, but no marked differences in sexual function were detected. Our results indicate that larger bracts, with a higher resource cost of maintenance, may create a trade-off that reduces their reproductive success. In contrast, the lower investment required by smaller bracts suggests more efficient resource allocation to fruit production, resulting in higher fruit set and fruit area.

AUTHOR CONTRIBUTIONS

JSA and RT planned and designed the research. Data collection was done by JSA and RT. Analysis and figures were done by JSA and RT, writing of the manuscript was done by JSA and RT. All authors read, provided suggestions, and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

SUPPORTING INFORMATION

Additional supporting information may be in the Supporting Information section at the end of the article.

REFERENCES

- Andersson, S. (1996) Flower-fruit size allometry at three taxonomic levels in *Crepis* (Asteraceae). *Biological Journal of the Linnean Society*, **58**, 401–407.
- Arista M., Ortiz P.L. (2007) Differential gender selection on floral size: an experimental approach using *Cistus salvifolius*. *Journal of Ecology*, **95**, 973–982.
- Armbruster W.S., Antonsen L., Pélabon C. (2005) Phenotypic Selection on Dalechampia Blossoms: Honest Signaling Affects Pollination Success. *Ecology*, **86**, 3323–3333.
- Barrett S.C.H. (1992) The Application of Sex Allocation Theory to Heterostylous Plants. In: Barrett S.C.H. (Ed.), *Evolution and Function of Heterostyly*. Springer, Berlin Heidelberg, 209–221.
- Barrett S.C.H. (2002) Sexual interference of the floral kind. *Heredity*, **88**, 154–159.
- Barrett S.C.H. (2019) ‘A most complex marriage arrangement’: recent advances on heterostyly and unresolved questions. *New Phytologist*, **224**, 1051–1067.
- Bookstein F.L. (1991) Morphometric tools for landmark data. Cambridge University Press, New York.
- Brys R., Jacquemyn H. (2010) Floral display size and spatial distribution of potential mates affect pollen deposition and female reproductive success in distylous *Pulmonaria officinalis* (Boraginaceae). *Plant Biology*, **12**, 597–603.
- Cardoso J.C.F., Viana M.L., Matias R., Furtado M.T., Caetano A.S., Consolaro H., Brito V.L.G. (2018) Towards a unified terminology for angiosperm reproductive systems. *Acta Botanica Brasiliica*, **32**, 329–348.
- Charlesworth D. (1989) Allocation to male and female function in hermaphrodites, in sexually polymorphic populations. *Journal of Theoretical Biology*, **139**, 327–342.

- Citerne H., Jabbour F., Nadot S., Damerval C. (2010) The Evolution of Floral Symmetry. In: *Advances in Botanical Research*. Elsevier, pp 85–137.
- Coelho C.P., Barbosa A.A.A. (2004) Biologia reprodutiva de *Psychotria poeppigiana* Mull. Arg. (Rubiaceae) em mata de galeria. *Acta Botanica Brasilica*, **18**, 481–489.
- Crawley M.J. (2013) *The R book*. Wiley, Chichester, UK.
- Da Cunha N.L., Aizen M.A. (2023) Pollen production per flower increases with floral display size across animal-pollinated flowering plants. *American Journal of Botany*, **110**, e16180.
- Darwin C. (1887) Concluding remarks on heterostyled plants. In: *The different forms of flowers on plants of the same species*. John Murray, London.
- Dunn P.K., Smyth G.K. (2018) *Generalized linear models with examples in R*. Springer, New York, USA, pp 573.
- Farré-Armengol G., Filella I., Llusia J., Peñuelas J. (2013) Floral volatile organic compounds: Between attraction and deterrence of visitors under global change. *Perspectives in Plant Ecology, Evolution and Systematics*, **15**, 56–67.
- Flora e Funga do Brasil (2026) Jardim Botânico do Rio de Janeiro. Available at: <http://floradobrasil.jbrj.gov.br/>
- Fox J., Weisberg S., Price B., Adler D., Bates D., BaudBovy G. (2018) Car: Companion to Applied Regression. R package version 3.1.0. <https://cran.r-project.org/web/packages/car>
- França-Neto J.D.B., Krzyzanowski F.C. (2022) Use of the tetrazolium test for estimating the physiological quality of seeds. *Seed Science and Technology* **50**:31–44.
- Glaettli M., Barrett S.C.H. (2008) Pollinator responses to variation in floral display and flower size in dioecious *Sagittaria latifolia* (Alismataceae). *New Phytologist*, **179**, 1193–1201.
- Goulson D. (1999) Foraging strategies of insects for gathering nectar and pollen, and implications for plant ecology and evolution. *Perspectives in Plant Ecology, Evolution and Systematics*, **2**, 185–209.
- Harder L.D., Barrett S.C.H. (1995) Mating cost of large floral displays in hermaphrodite plants. *Nature*, **373**, 512–515.
- Hartig F., Lohse L. (2022) DHARMA: residual diagnostics for hierarchical (multi-level/mixed) regression models. R package version 0.4.5. <https://CRAN.Rproject.org/package=DHARMA>
- Karron J.D., Mitchell R.J. (2012) Effects of floral display size on male and female reproductive success in *Mimulus ringens*. *Annals of Botany*, **109**, 563–570.
- Keller B., Thomson J.D., Conti E. (2014) Heterostyly promotes disassortative pollination and reduces sexual interference in Darwin's primroses: evidence from experimental studies (G. Kudo, Ed.). *Functional Ecology* **28**, 1413–1425.
- Lloyd D.G., Barrett S.C.H. (Eds) (1996) *Floral Biology*. Springer US, Boston, MA.
- Lüdecke D. (2018) Ggeffects: Tidy data frames of marginal effects from regression models. *Journal of Open Source Software*, **3**, 772.
- Lunau K., Maier E.J. (1995) Innate colour preferences of flower visitors. *Journal of Comparative Physiology*, **177**, 1–19.

- Martins F.B., Gonzaga G., Dos Santos D.F., Reboita M.S. (2018) Classificação climática de Köppen e de Thornthwaite para Minas Gerais: cenário atual e projeções futuras. *Revista Brasileira de Climatologia*, **1**.
- Nicholls M.S. (1986) Population composition, gender specialization, and the adaptive significance of distyly in *linum perenne* (linaceae). *New Phytologist*, **102**, 209-217.
- Nishihiro J., Washitani I., Thomson J.D., Thomson B.A. (2000) Patterns and consequences of stigma height variation in a natural population of a distylous plant, *Primula sieboldii*. *Functional Ecology*, **14**, 502–512.
- Obeso, J.R. (2002) The costs of reproduction in plants. *New Phytologist*, **155**, 321-348.
- Ohashi K., Yahara T. (1999) How Long to Stay on, and How Often to Visit a Flowering Plant? *Oikos*, **86**, 2.
- Ohashi K., Yahara T. (2001) Behavioral responses of pollinators to variation in floral display size and their influences on the evolution of floral traits. In: Chittka L., Thomson J.D. (Eds), *Cognitive Ecology of Pollination*. Cambridge University Press; Cambridge, UK: 274–296.
- Ornelas J.F., Jiménez L., González C., Hernández A. (2004) Reproductive ecology of distylous *Palicourea Padifolia* (Rubiaceae) in a tropical montane cloud forest. I. Hummingbirds' effectiveness as pollen vectors. *American Journal of Botany*, **91**, 1052–1060.
- Pailler T., Humeau L., Figier J. Reproductive trait variation in the functionally dioecious and morphologically heterostylous island endemic *Chassalia corallioides* (Rubiaceae). *Biological Journal of the Linnean Society*, **64**, 297-313.
- Pannell J.R. (2018) Gender specialisation and stigma height dimorphism in Mediterranean *Lithodora fruticosa* (Boraginaceae) (J. Arroyo, Ed.). *Plant Biology*, **20**, 112–117.
- Pélabon C., Thöne P., Hansen T.F., Armbruster W.S. (2012) Signal honesty and cost of pollinator rewards in *Dalechampia scandens* (Euphorbiaceae). *Annals of Botany*, **109**, 1331–1339.
- Pickering C.M., Arthur J.M. (2003) Patterns of resource allocation in the dioecious alpine herb *Aciphylla simplicifolia* (Apiaceae). *Austral Ecology*, **28**, 566–574.
- Rohlf F.J. (2015) The tps series of software. *Hystrix*, **26**, 9–12.
- Rohlf F.J., Slice D. (1990) Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Biology*, **39**, 40–59.
- Sargent R.D., Goodwillie C., Kalisz S., Ree R.H. (2007) Phylogenetic evidence for a flower size and number trade-off. *American Journal of Botany*, **94**, 2059-2062.
- Schneider C.A., Rasband W.S., Eliceiri K.W. (2012) NIH image to ImageJ: 25 years of image analysis. *Nature Methods*, **9**, 671–675.
- Schoen D.J., Ashman T.-L. (1995) The evolution of floral longevity: resource allocation to maintenance versus construction of repeated parts in modular organisms. *Evolution*, **49**, 131–139.
- Song B., Chen J., Lev-Yadun S., Niu Y., Gao Y., Ma R., Armbruster W.S., Sun H. (2024) Multifunctionality of angiosperm floral bracts: a review. *Biological Reviews*, 000-000.

- Teixido A.L., Valladares F. (2013) Large and abundant flowers increase indirect costs of corollas: a study of coflowering sympatric Mediterranean species of contrasting flower size. *Oecologia*, **173**, 73-81.
- Thompson J.D. (2001) How do visitation patterns vary among pollinators in relation to floral display and floral design in a generalist pollination system? *Oecologia*, **126**, 386–394.
- Trevizan R., Caetano A.P.S., Brito V.L.G., Oliveira P.E., Telles F.J. (2023) Stamen and pollen heteromorphism linked to the division of labour in Melastomataceae species. *Flora*, **305**, 152315.
- Trevizan R., Oliveira P.E., Brito V.L.G., Oliveira L., Telles F. (2025) Bract size affects resource availability and fruit set in a hummingbird-pollinated plant with distyly polymorphism. *Plant Biology*, **27**, 1478–1487.
- Trevizan R., Oliveira P.E., Cardoso J.C.F. (2024) Investigating the longstanding mystery of stigma length differences between morphs of distylous plants. *Plant Biology*, **26**, 421–426.
- Valois-Cuesta H. (2009) Reproductive ecology of *Psychotria poeppigiana* (Rubiaceae): a comparative analysis between long-styled and short-styled plants. *Ecotrópicos*, **22**, 1-12.
- Valois-Cuesta H., Soriano P.J., Ornelas J.F. (2012) Gender specialization in *Palicourea demissa* (Rubiaceae), a distylous, hummingbird-pollinated treelet. *Plant Systematics and Evolution*, **298**, 975–984.
- Van Der Kooi C.J., Dyer A.G., Kevan P.G., Lunau K. (2019) Functional significance of the optical properties of flowers for visual signalling. *Annals of Botany*, **123**, 263–276.
- Van Der Niet T., Johnson S.D. (2012) Phylogenetic evidence for pollinator-driven diversification of angiosperms. *Trends in Ecology & Evolution*, **27**, 353–361.
- Waser N.M. (1983) The Adaptive Nature of Floral Traits: Ideas and Evidence. In: Real L. (Eds), *Pollination Biology*. Academic Press, Inc.; London, UK: 241–285.
- Watanabe K., Shimizu A., Sugawara T. (2014) Dioecy derived from distyly and pollination in *Psychotria rubra* (Rubiaceae) occurring in the Ryukyu Islands, Japan. *Plant Species Biology*, **29**, 181–191.
- Weiner, J. (2004) Allocation, plasticity and allometry in plants. *Perspectives in Plant Ecology, Evolution and Systematics*, **6/4**, 207-215.
- Wright G.A., Schiestl F.P. (2009) The evolution of floral scent: the influence of olfactory learning by insect pollinators on the honest signalling of floral rewards. *Functional Ecology*, **23**, 841–851.
- Zelditch M.L., Swiderski D.L., Sheets H.D. (2012) Geometric morphometrics for biologists: A primer. Academic Press, London, UK, pp 475.

FIGURE CAPTIONS

Figure 1. Differences between L-styled and S-styled floral morphs and (A) bract size, (B) flower length (mm), and (C) fruit area (mm). Red dots (L-styled) and blue dots (S-styled).

Figure 2. Relationship between pollen deposition on stigmas and floral morphs (L-styled and S-styled). Red dots (L-styled) and blue dots (S-styled).

Figure 3. Negative effect of bract size (predictor variable) on (A) fruit-set proportion and (B) fruit area (mm). The points correspond to the raw data. The line indicates the plotted model, and the purple bands indicate the 95% confidence interval.

Figure 4. Positive effect of bract size (predictor variable) on bract mortality. The line indicates the plotted model, and gray bands indicate the 95% confidence interval. The points correspond to the raw data.

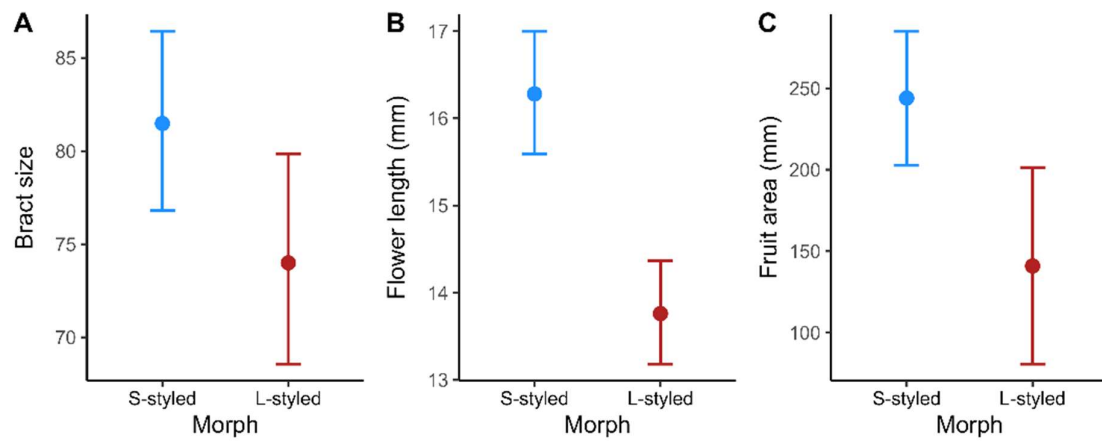


Figure 1. Differences between L-styled and S-styled floral morphs and (A) bract size, (B) flower length (mm), and (C) fruit area (mm). Red dots (L-styled) and blue dots (S-styled).

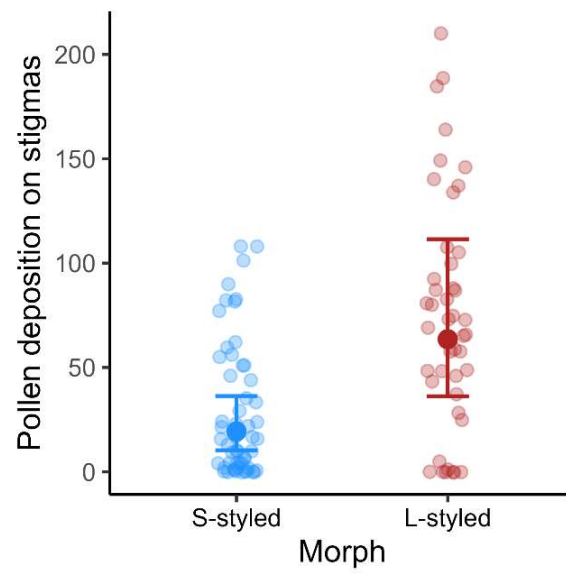


Figure 2. Relationship between pollen deposition on stigmas and floral morphs (L-styled and S-styled). Red dots (L-styled) and blue dots (S-styled).

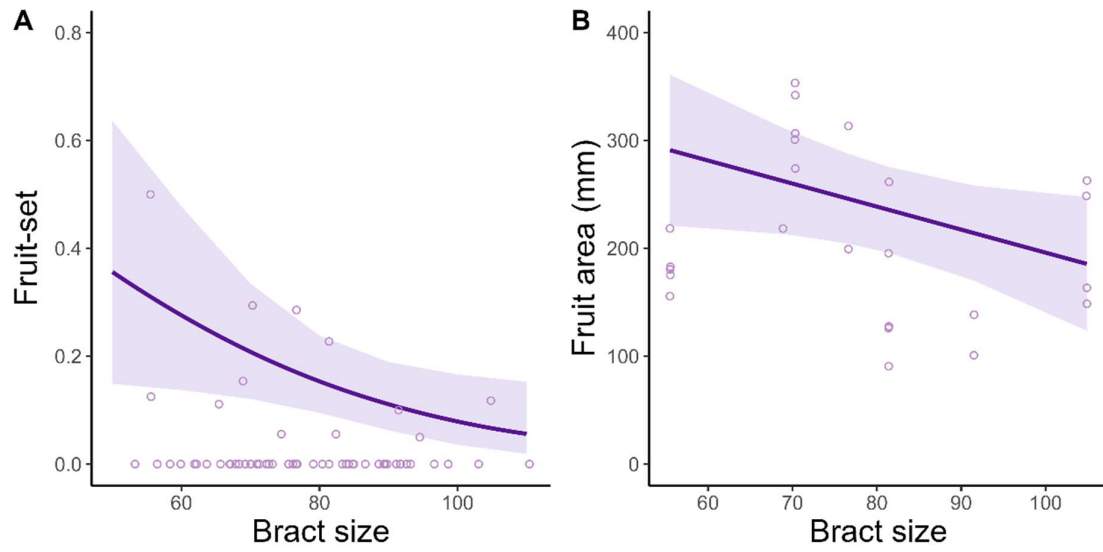


Figure 3. Negative effect of bract size (predictor variable) on (A) fruit-set proportion and (B) fruit area (mm). The points correspond to the raw data. The line indicates the plotted model, and the purple bands indicate the 95% confidence interval.

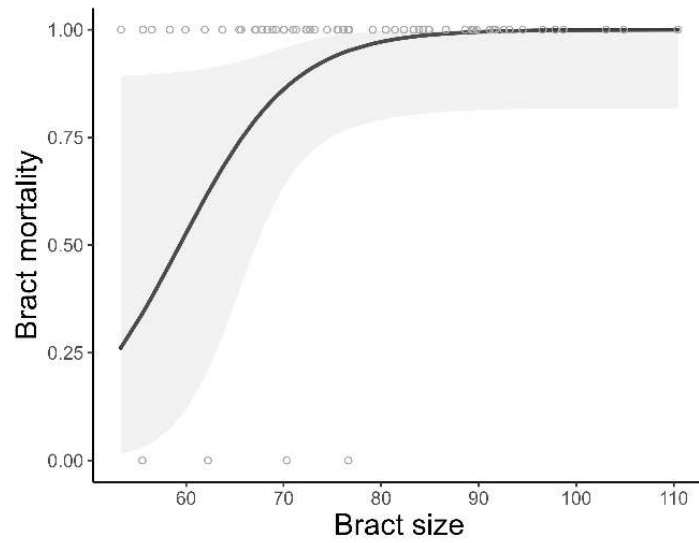


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SUPPORTING INFORMATION

Table S1. Details of models concerning the effects of bracts size and morph type on fruit and seed set, including the model parameters and their respective results.

Model parameters			Results		
Response variable	Family (link)	Predictor variables	χ^2	df	p
Fruit area (mm ²)	<i>Gaussian</i> (identity)	Bract size	3,87	1	0,04
		Morph type	7,177	1	0,007
Fruit perimeter (mm)	<i>Gaussian</i> (identity)	Bract size	1,06	1	0,3
		Morph type	3,54	1	0,059
Seed area (mm ²)	<i>Gaussian</i> (identity)	Bract size	2,49	1	0,11
		Morph type	1,335	1	0,24
Seed perimeter (mm)	<i>Gaussian</i> (identity)	Bract size	0,01	1	0,91
		Morph type	2,77	1	0,09
Seed weight (g)	<i>Gaussian</i> (identity)	Bract size	1,86	1	0,17
		Morph type	0	1	0,99
Seed germination (presence/absence)	<i>Binomial</i> (logit)	Bract size	1,83	1	0,17
		Morph type	0,87	1	0,34