Brazilian Journal of Botany
https://doi.org/10.1007/s40415-024-01032-9

REPRODUCTIVE BIOLOGY - ORIGINAL ARTICLE

# Resource distribution and reproductive efficacy of dimorphic stamens of *Senna occidentalis* (L.) Link: a case study on the division-of-labour hypothesis

**Suman Paul**[1] · **Bornali Gogoi**[1] · **Badal Kumar Datta**[1] · **Bhushan B. Dholakia**[2]

Received: 25 November 2023 / Revised: 1 July 2024 / Accepted: 11 July 2024

**Abstract**

Heteranthery, the presence of different sets of stamens in the same flower, reduces the conflict between effective pollinators and flowers by ensuring adequate resources for the pollinators without affecting reproductive success. Here, we tested the division-of-labour hypothesis in *Senna occidentalis*, a buzz-pollinated, self-compatible, heterantherous species. The fecundity of pollen grains from long and short stamens was evaluated using hand cross-pollination experiments and by comparing the quantity, size, and quality of pollen in different sets of stamens. The visitation rate and behaviour of floral visitors were also assessed, while their preferences for different sets of stamens were checked using stamen exclusion experiments. The long-pollinating stamens produced a greater number and volume of pollen grains compared to the short-feeding stamens. The pollen grains from long stamens exhibited better germination rates with higher production of fruit and seeds than short stamen pollens in cross-pollination experiments. Nine floral visitors foraged on the flowers, and the large carpenter bees (*Xylocopa* sp.) were the most dominant and effective pollinators. The long stamens deposited pollen on the lateral and ventral thorax of large-sized pollinators, while the short stamens placed pollen on the ventral and abdomen portions. Therefore, the short stamens might produce pollen grains primarily to feed their pollinators, while the long stamens might generate pollen for pollination and successful fertilization. Further, breeding system experiments indicated a significant pollen limitation and dependency on insect pollination for the increased reproductive outputs (fruit and seed sets). Overall, our findings could indicate a differential distribution of pollen grains in long and short stamens. The differences in the quality and quantity of pollen grains produced by dimorphic stamens of *S. occidentalis* could fit the division-of-labour hypothesis.

**Keywords** Anther dimorphism · Division-of-labour · Heteranthery · Pollen fecundity · Reproductive success · *Senna occidentalis*

## 1 Introduction

Pollen transfer by animal vectors is a key process in plant reproduction and the evolution of angiosperms. Plants have evolved diverse strategies to attract and reward their pollinators while ensuring efficient reproductive success (Barrett 2010). Flowering plants exhibit great diversity in the morphology of reproductive organs, especially the male structures (i.e. stamens). Variations in male structures were evident among several closely related species, between populations, and also within and among flowers of the same species (Endress 1996; Barrett 2010). Among them, stamen polymorphism within a flower is relatively uncommon. Stamen polymorphism is known as heteranthery and involves the presence of two (dimorphic) and/or more distinct sets of stamens in a single flower with potentially contrasting

---

✉ Suman Paul
sumanoxy1993@gmail.com

✉ Bhushan B. Dholakia
bhushan1410@yahoo.com

Bornali Gogoi
bornaligogoi.bg19@gmail.com

Badal Kumar Datta
dattabadal2008@gmail.com

[1] Plant Taxonomy and Biodiversity Laboratory, Department of Botany, Tripura University, Suryamaninagar, Tripura 799022, India

[2] MBB Department, Tripura University, Suryamaninagar, Tripura 799022, India

functions (Barrett 2010; Paulino et al. 2016). Heteranthery is rare but occurs in taxonomically diverse groups and in a variety of forms, which might have evolved through several independent events during the evolutionary history of flowering plants (Graham and Barrett 1995; Barrett 2010). It occurs in at least 12 orders representing 16 families across angiosperms and is commonly found in the family members of Leguminosae, Pontederiaceae, Melastomataceae, Solanaceae, Commelinaceae, etc. (Barrett 2010; Vallejo-Marín et al. 2010; Papaj et al. 2017; Basso-Alves et al. 2022).

Heterantherous flowers produce stamens that differ in colour, size, shape, pollen production, pollen shape, and size, and thus, pollinators are attracted to them (Vallejo-Marín et al. 2009; Solís-Montero et al. 2018). Flowers with two sets of stamens are the most common, and anthers are clearly distinguishable. The first set is centrally placed in the flowers, which are composed of bright (usually yellow)-coloured stamens that are short in length. The second set of anthers is placed away from the central axis of the flower and is often cryptically coloured. These stamens are larger compared to the first set of stamens (Vallejo-Marín et al. 2009). A third set of stamens (medium-sized) is also found in some members of Solanaceae (*Solanum* spp.), Leguminosae (*Senna* spp.), etc. (Lou et al. 2009; Mesquita-Neto et al. 2017). The flowers of *Senna* spp. (Leguminosae), *Commelina* spp. (Commelinaceae), etc. contain an additional fourth set of sterile stamens, known as staminodes (Barrett 2010; Mesquita-Neto et al. 2017; Pinheiro-Costa et al. 2018). Heteranthery is commonly associated with floral characters, and their effective pollinator traits have evolved to enhance reproductive success (Barrett 2010). The flowers exhibiting heteranthery with poricidal anthers are pollinated mainly by bees that extract pollen from anthers by producing vibration, and this phenomenon is known as buzz-pollination (Michener 1962; Vogel 1978; Buchmann 1983). Buzz-pollinated flowers release the pollen grains through small apical pores (also known as poricidal anther dehiscence) as a result of vibration during the visits of floral visitors (Buchmann 1983). In these flowers, pollen plays a dual and conflicting role as a protein-rich food source for the pollinators and as a reproductive agent for successful fertilisation (Endress 1996). This conflict has been commonly referred to as the "pollen dilemma," which applies to all bee-pollinated flowers with pollen as the only resource for their effective pollinators (Endress 1996). Moreover, the consumption of fertile pollen does not consequently decrease reproductive output (Mesquita-Neto et al. 2017). The 'division-of-labour' hypothesis involved the presence of two or more sets of morphologically different stamens in a single flower (Müller 1883; Darwin 1887). This hypothesis has been empirically investigated in different heterantherous genera, such as *Solanum* (Papaj et al. 2017; Vallejo-Marín et al. 2022), *Senna* (Amorim et al. 2017; Pinheiro-Costa et al. 2018; Dellinger et al. 2019), *Cassia* (Saab et al. 2021), *Chamaecrista* (Nogueira et al. 2018), *Rhynchanthera* (Konzmann et al. 2020), *Microlicia* (Velloso et al. 2018), and *Clarkia* (Kay et al. 2020). This differential allotment of resources among stamens might ensure adequate assets for their effective pollinators without considerably affecting the availability of fertile male gametes for successful fertilisation and reproductive accomplishment (Vallejo-Marín et al. 2009; Lunau et al. 2015).

Not all the visitors to buzz-pollinated flowers are pollinators. The effectiveness of floral visitors and their contribution to plant reproductive success might vary greatly depending on a set of morphological and behavioural components, including body size, frequency of visits, duration of visits, and frequency of stigma contact (Ne´eman et al. 2009; Mesquita-Neto et al. 2017, 2018; Solís-Montero and Vallejo-Marín 2017; Konzmann et al. 2020). The body size of floral visitors is an important trait that can determine their effectiveness in contacting the floral reproductive organs during visits, which could have implications for promoting fruit and seed sets (Vivarelli et al. 2011; Lazaro et al. 2013). Large and medium-sized bees were reported to be highly effective and resulted in higher reproductive success (Lou et al. 2008; Liu and Pemberton 2009). However, small-sized bees may exhibit low effectiveness in buzz-pollinated flowers and are capable of destroying the anthers while collecting pollen, which can further decrease the chances of pollen dispersal by other effective pollinators and thus, eventually lead to a reduction in reproductive success (Renner 1983; Solís-Montero et al. 2015). However, very few studies have documented the morphological and behavioural traits that could distinguish between effective (legitimate) pollinators and pollen thieves and/or illegitimate visitors in pollen-only buzz-pollinated flowers (Renner 1983; Solís-Montero et al. 2015; De Luca and Vallejo-Marín 2013; Mesquita-Neto et al. 2017). The genus *Senna* (Cassiinae, Leguminosae) consists of about 350 species with a pantropical distribution. The flowers are mostly nectarless with a shiny yellow colour and are pollinated by pollen-collecting bees (Buchmann 1974; Dulberger 1981). They exhibit a diverse array of floral traits, including heteranthery, poricidal anthers, enantiostyly, point-tipped stigmas, asymmetrical corolla, and androecia (Irwin and Barneby 1981; Gottsberger and Silberbauer-Gottsberger 1988; Owens and Lewis 1989; Dulberger et al. 1994; Marazzi et al. 2008). *Senna occidentalis* (L.) Link, a short-lived annual or biennial shrub with a 0.5–2.5 m height, has a pantropical distribution (Prota 2016). The weed usually grows in grasslands, roadsides, open woodlands, pastures, coastal areas, disturbed areas, and wastelands (Lum Nde et al. 2021). It has been reported as an invasive species in countries including Kenya, Uganda, Cuba, Tanzania, and regions of Asia and Oceania (Oviedo Prieto et al. 2012; Bionet-Eafrinet 2016; Pier 2016). The flowers of this species exhibit stamen dimorphism, which consists of two long and four short stamens. Thus, we have chosen *S. occidentalis* (Leguminosae) to test the division-of-labour hypothesis among different sets of stamens, reflecting the extensive occurrence of heteranthery in the family. We anticipated that like other pollen flowers (i.e., flowers that produce pollen as the only resource for their pollinators), the species might exhibit a differential distribution of pollen resources among different sets of stamens. We hypothesised that pollinators might get attracted more to the short stamens with feeding functions, while the long stamens might contribute to successful reproduction by producing pollen grains with greater fecundity. The present study was designed to answer the following questions: (1) Does the size, quantity, quality, and morphology of pollen vary among different sets of stamens? (2) Are there any variations in the fecundity of pollen from different sets of stamens? (3) What are the effective pollinators of *S. occidentalis*? (4) Which stamens are recognised as feeding sources, and do all visitors exhibit the same preference?

## 2 Materials and methods

**Study area and climate** – The present study was conducted on a natural population of *Senna occidentalis* near the municipal dumping ground area (23° 46′ 55″ N; 91° 15′ 11″ E, 19 MSL), Agartala, Tripura, India. The climate at our study site is tropical monsoon with mean annual rainfall ranges from 2250 to 2500 mm. The temperature of the study site ranges from 10 to 36° C. Field observations and hand pollination experiments were conducted during the peak flowering period of the species (August–October 2021), which corresponded to the monsoon and the post-monsoon seasons. The study site is dominated by *S. occidentalis* plants along with some co-occurring species such as *Solanum sisymbriifolium* Lam., *Lantana camara* L., *Sida acuta* Burm. fil., *Parthenium hysterophorus* L., and *Chromolaena odorata* (L.) RM King & H Rob.

**Floral anthesis and morphometry** – Floral phenology was observed in two inflorescences from each of five randomly selected plants. The flowers were observed from the first day of anthesis until the corolla wilted. A total of 15 flowers were collected randomly from five different individuals, and several floral morphological traits (e.g., petal length, petal width, style length, stigma diameter, stigma length, ovary length, ovary width, sepal length, and sepal width) were recorded. We used a Student's *t*-test to assess the differences among different sets of stamens (Luo et al. 2009).

**Pollen morphology and quantity** – To estimate the quantity of pollen grains produced by dimorphic stamens of *S. occidentalis*, 20 mature floral buds were collected from 10 different individuals (two buds per plant). The medium-sized stamens were excluded from these experiments as they did not produce fertile pollen grains. Using a glass rod, anthers were individually crushed in different 1.5 mL Eppendorf tubes with a 1 mL solution of distilled water and glycerin (9:1 ratio) and a few drops of acetocarmine stain. The pollen suspension was vortexed for 1 min. and 10 µL of this suspension was transferred onto a microscopic slide with a micropipette. All pollen grains were covered with a cover glass and were counted. This procedure was repeated five times. The total number of pollen grains was calculated by multiplying the average pollen count in the 10 µL volume of suspension by 100 (Shivanna and Rangaswamy 1992; Shivanna and Tandon 2014). A total of 20 mature flower buds were collected randomly from different individuals ($N = 10$). The number of ovules from every flower was counted under a dissecting stereo microscope (Stemi 508, Carl Zeiss, Germany). The pollen-to-ovule ratio was then estimated by dividing the pollen grain numbers by the ovule numbers per flower (Cruden 1977).

– To test for differences in volume of pollen grains between long and short anthers, the polar and equatorial diameter of 50 non-acetolyzed pollen grains were measured for each set of stamens with an optical microscope (Carl Zeiss AXIO Lab A1, Germany) having a digital camera (AxioCamERc 5s). The pollen grain values were adjusted to a known geometric shape (oblate-spheroidal), and the pollen volume was calculated using the following equation: $v = (4/3)\,\pi a^2 b$, where $v$ was the volume of pollen (µm$^3$), $a$ was the radius of the polar diameter, and $b$ was the radius of the equatorial diameter. To estimate the differences between the volume and quantity of pollen grains in two sets of stamens, the analysis of variance with a generalised linear model (GLM-ANOVA with Gaussian distribution) was used in R statistical software ver. 4.2.1 (R Core Team 2022).

**Pollen fecundity** – The fecundity of different sets of stamens were evaluated using a pollen stainability test and in vitro pollen germination techniques, which have been widely used to assess pollen vigour (Shivanna and Tandon 2014). To test if there was any difference in pollen viability among different sets of stamens, 40 fresh open flowers were collected from 10 different individual plants (four flowers each). Pollen viability was estimated using the two staining techniques: (1) Acetocarmine staining solution, where the pollen grain was considered viable if it was in dark pink; (2) Aniline blue–lactophenol staining solution, where the blue colour of pollen grain indicated its viability (Kearns and Inouye 1993; Khatun and Flowers 1995). In both staining techniques, the pollen grains were considered non-viable if they remained unstained and were easily distinguishable from viable pollen grains with stains. Each set of stamens was placed separately in a 1.5 mL centrifuge tube containing one of the two staining solutions and incubated for 3–5 min. at room temperature (25–30 °C). Pollen grains were observed using a compound microscope (Carl Zeiss AXIO Lab A1, Germany). We counted the viable and non-viable pollen grains in each field of view for a total count of no less than 50 pollen grains. The effects of different stains on pollen viability were analysed using GLM-ANOVA (Gaussian distribution and identity link function). We used Tukey's post hoc test to compare the significant differences among treatments. The percentage viability values were log-transformed before building the GLM model to meet the normal distribution. We used Shapiro–Wilk's test to determine the distribution of the data set (Mishra et al. 2019).

– Differences in pollen viability and physiology among different sets of anthers were analysed using the in vitro pollen germination method (Dafni and Firmage 2000). Germination tests were performed using pollen from 10 fresh flowers collected from five different individuals (two flowers per plant). Pollen grains were placed in Brewbaker-Kwack (BK) medium (Brewbaker and Kwack 1963), and supplemented with different concentrations (10–40%) of sucrose. The BK medium with pollen grains was incubated for 20 h at room temperature (25–30 °C) in an ELISA plate with one sample per well. The number of germinated or non-germinated pollen grains was counted (100 pollen grains per set of anther/flower) with a light microscope (Olympus CX21i, Japan). Pollen grains were considered germinated if the pollen tube length was greater than the diameter of the pollen (Chen and Fang 2016). The differences in the number of germinated pollen grains in each set of stamens were compared using the GLM-ANOVA procedure (Gaussian distribution and identity link function) using the stats package in R ver. 4.2.1. Tukey's *post-hoc* test was used to differentiate the germination ability of pollen grains from different sets of stamens.

**Breeding system** – To estimate the reproductive fecundity of different sets of *S. occidentalis* anthers, breeding system experiments were performed. Mature flower buds were emasculated and bagged in the afternoon. The next day, bags were opened, and flowers were pollinated with pollen (200–300 pollen grains per stigma) from long stamens (76 flowers) and short stamens (80 flowers) and re-bagged. To test the degree of spontaneous self-pollination (autogamy), flower buds were bagged and observed till fruit and seed set. Another 20 inflorescences from different individual plants were tagged and kept open for natural pollination. To test the ability of apomixis, 56 mature flower buds (N = 16 plants) were emasculated and bagged to restrict pollen deposition. Further, we tested the efficacy of pollinators, in which 86 mature flower buds (N = 20 plants) were emasculated and kept open for natural pollination. To assess the magnitude of pollen limitation for the studied species, 56 freshly opened flowers (N = 16 plants) were supplemented with cross pollen collected from fresh flowers at least 10 m away from the target plants. Geitonogamous self-pollination potential was also evaluated, where 81 flower buds (N = 20 plants) were emasculated and pollinated using pollen grains collected from other flowers of the same individuals. The fruit set (number of fruits produced/ number of flowers submitted to each treatment) and seed set (number of seeds produced per fruit) of different pollination treatment groups were compared. We used Shapiro–Wilk's test (Konzmann et al. 2020) to evaluate the normal distribution of the data. The percentage fruit set data was log-transformed to meet the normal distribution. The statistical significance of the fruit sets and seed sets under different treatments was compared using GLM-ANOVA (using a Gaussian distribution). Tukey's post hoc test was used to assess the differences among pollination treatments.

**Floral visitors** – To evaluate the floral visitation frequency, focal observations were performed on different individuals on clear, sunny days. These observations were carried out for seven days between 7:00 a.m. to 2:00 p.m. (7 h), with 49 h in total. All the pollinators were collected and/or photographed for identification. Voucher specimens of pollinators (TU/Dipt/Diop-7834; TU/Dipt/Diop-7838) were archived at the Department of Zoology, Tripura University. Floral visitors were noted as pollinators if they came into contact with stigma and anthers. Floral visitation frequency was calculated using the following equation (Shivanna and Tandon 2014; Paul et al. 2024):

$$\text{Visitation frequency} = \frac{\text{Number of pollinator visits}}{\text{Number of open flowers} * \text{Observation time}}$$

**Floral choices of the visitors** – From the survey of floral visitors, *Xylocopa* bees were documented as being the dominant pollinator of *S. occidentalis*. Therefore, to evaluate the role of different sets of anthers (pollinating and feeding anthers) in attracting *Xylocopa* bees, flowers were experimentally manipulated with different treatments (stamen exclusion). The experiments were conducted on randomly selected individuals (N = 23 plants) of *S. occidentalis*. In these individuals, 46 inflorescences comprising 274 flowers were tagged. These inflorescences were assigned to six different treatment groups, and all the flowers of the same inflorescence received one of the six treatments. Flowers of each inflorescence were assigned to one of the following treatments: (a) Intact flowers as control (unmanipulated), (b) removal of long stamens, (c) removal of medium stamens, (d) removal of short stamens, (e) removal of staminodes, and (f) removal of all types of stamens and staminodes. Mature flower buds were emasculated in the afternoon one day before the opening and bagged. Bags were removed the next morning before the observation were started. The numbers of control and test flowers were maintained nearly equal in all the above-mentioned experimental groups. All the experiments were repeated at least three times on randomly selected individuals (N = 23 plants). Focal observation on each plant was carried out for a total of 60 min. [divided into four blocks of 15 min. each with 10 min. of gaps]. We recorded the behaviour of bees and their floral choices (controlled or manipulated flowers). The visitation frequency of pollinators was calculated as the total number of visits per flower per hour. The observations were carried out during peak visiting hours (recorded during observations) for five days, from 8:00 a.m. to 12:00 p.m., totalling 20 h. The statistical significance of floral choices by pollinators under different treatments was compared using Tukey's post hoc test after building a GLM model using Gaussian distribution and identity link function (Mesquita-Neto et al. 2021; Paul et al. 2024). Shapiro–Wilk's test was used to assess the normal distribution of the data. All the statistical analyses were performed using different packages implemented in R statistical software ver. 4.2.1 (R Core Team 2022).

## 3 Results

**Flowering phenology and morphology varied among different stamens** – The flowering period of *S. occidentalis* at the study site (Fig. 1a) lasted from July to January of the next year. The peak flowering period occurred between September and October. Inflorescences were paniculate. The flowers were yellow, bisexual, and zygomorphic to slightly asymmetric. The flowers consisted of three dissimilar sets of stamens, including two long lateral stamens, one medium-sized stamen (which was mostly sterile and did not produce fertile pollen grains), and four short central stamens (Fig. 1b). There were also three dorsal sterile staminodes. Flowers remained open from 6:00 a.m. to 2:00 p.m. and closed completely by 3:00 p.m. The life span of flowers was mostly two days, and the petals fell off afterward. Each inflorescence produced 24.67 ± 6.25 (mean ± SD) (N = 15) flower buds, and 3.53 ± 1.09 (N = 15) flowers per inflorescence that opened each day.

– The diameter and length of the flowers ranged from 20.8 to 34.5 mm (28.58 ± 4.29) and 25.20 to 34.90 mm (30.66 ± 3.58), respectively. The lengths of the filament and anther of the short-sized stamen were 4.08 ± 1.01 and 3.52 ± 0.86, respectively. It was significantly shorter than the filament of the long (5.48 ± 0.65) stamen (*t*-test, $t = 17.29$, $df = 88$, *p*-value < 0.001). Similarly, anther lengths of the long stamen (7.74 ± 1.44) and short stamen (3.52 ± 0.86) were significantly different (*t*-test, $t = 6.89$, $df = 88$, *p*-value < 0.001). The lengths of the filament and anther of medium-sized stamen were 2.21 ± 0.69 and 3.14 ± 1.24, respectively. The statistical analysis of floral morphometric characters is presented in Table 1.

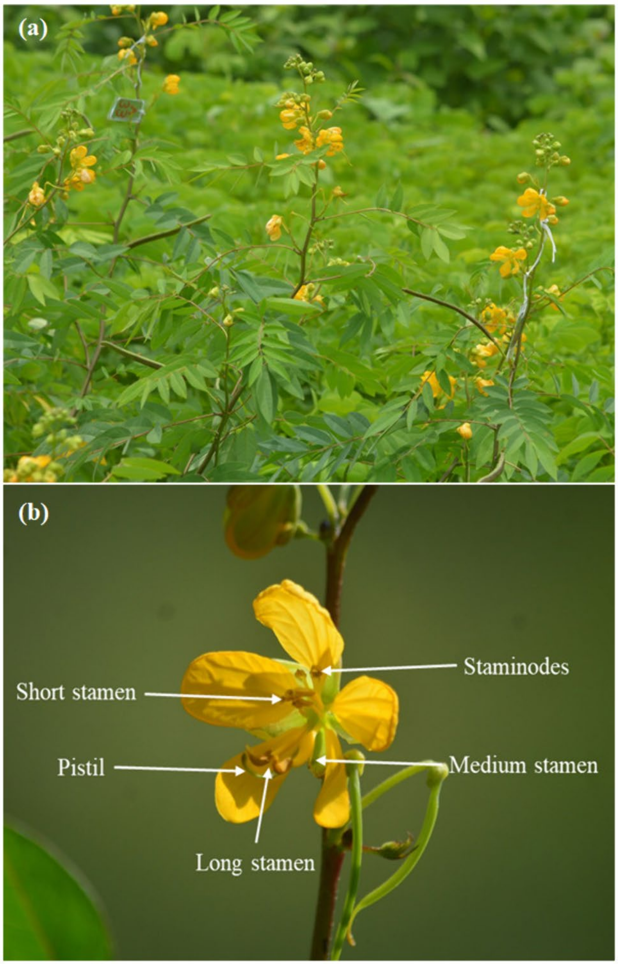

**Fig. 1** Natural population of *S. occidentalis* at a field site. **a** Flowering individuals and **b** a closer view of floral morphology

**Identical pollen morphology, but variation in pollen quantity from different types of stamens** – Pollen grains of *S. occidentalis* were sub-oblate in shape with reticulate exine ornamentation at 400X magnification under the light microscope. There were no differences in the morphology, ornamentation, size, or shape of pollen grains that were produced by short and long stamens. Further, both sets of stamens generated relatively similar volumes of pollen grains. On average, the volume of a single pollen grain produced by short stamens was 46,085.95 ± 5320.11 $\mu m^3$ (mean ± SD) and long stamens was 44,829.95 ± 4700.11 $\mu m^3$ (Supplementary Fig. S1). Subsequently, there were no significant differences in the volume of single pollen grains produced by both sets of stamens (GLM, $df = 1$, F value = 1.534, *p*-value = 0.218). The single long anther produced a higher volume of pollen compared to a single short one (Fig. 2a; GLM, $df = 1$, F

**Table 1** Statistical analysis of floral morphological characters of *S. occidentalis*

| Character | | Sample size (N) | Range (mm) | Mean (mm) | SD (mm) | C.V. |
|---|---|---|---|---|---|---|
| Petal length | | 75 | 12.0–22.0 | 16.00 | 2.18 | 0.13 |
| Petal width | | 75 | 4.0–17.5 | 8.78 | 2.88 | 0.32 |
| Style length | | 15 | 11.0–17.0 | 14.12 | 1.73 | 0.12 |
| Stigma diameter | | 15 | 0.5–6.0 | 1.13 | 1.35 | 1.19 |
| Stigma length | | 15 | 0.7–1.5 | 0.98 | 0.21 | 0.22 |
| Ovary length | | 15 | 1.4–4.0 | 2.38 | 0.77 | 0.32 |
| Ovary width | | 15 | 1.0–4.0 | 2.52 | 0.99 | 0.39 |
| Sepal length | | 75 | 5.0–11.6 | 8.45 | 1.54 | 0.18 |
| Sepal width | | 75 | 3.0–9.0 | 5.55 | 1.22 | 0.22 |
| Long stamen | Anther | 30 | 5.0–10.0 | 7.74 | 1.44 | 0.18 |
| | Filament | 30 | 4.3–7.0 | 5.48 | 0.65 | 0.11 |
| Medium stamen | Anther | 15 | 0.9–6.0 | 3.14 | 1.24 | 0.39 |
| | Filament | 15 | 1.0–3.5 | 2.21 | 0.69 | 0.31 |
| Short stamen | Anther | 60 | 2.0–5.5 | 3.52 | 0.86 | 0.24 |
| | Filament | 60 | 1.5–5.1 | 4.08 | 1.01 | 0.24 |
| Staminode length | | 45 | 2.0–6.5 | 4.28 | 1.28 | 0.24 |

C.V., coefficient of variation

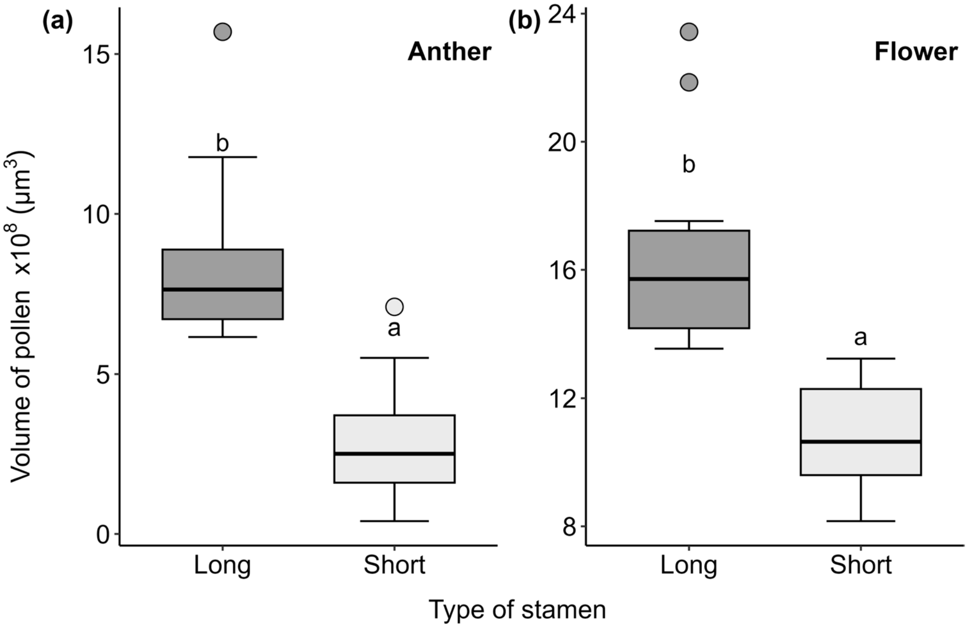

**Fig. 2** Median and percentiles (25–75%) of the **a** volume of pollen per anther and **b** flower (µm$^3$) from long and short stamens of *S. occidentalis* (N = 50). Statistical analysis: volume of pollen per anther (GLM: F = 123.6, $p < 0.001$) and per flower (GLM: F = 23.61, $p < 0.001$; Gaussian distribution). Different letters represented significant differences at a $p$-value < 0.05

value = 123.6, $p$-value < 0.0001). Considering both sets of stamens of a flower, 60.68% of pollen volume was allotted to long stamens and 39.32% to short stamens (GLM, $df = 1$, F value = 23.61, $p$-value < 0.0001) (Fig. 2b).

– The single long stamen produced more pollen grains (18,548.50 ± 5139.76) than a single short stamen (5852.75 ± 3075.95) (GLM, $df = 1$, F value = 136.5, $p$-value < 0.0001) (Fig. 3a). Considering the total sets of stamens in a flower, on average, 61.33% of pollen grains were allotted to long stamens and 38.67% to short stamens (GLM, $df = 1$, F value = 26.33, $p$-value < 0.0001) (Fig. 3b). The ovary (N = 10) produced on average 80.80 ± 10.54 ovules, and the pollen-to-ovule ratio was 756.02 ± 85.89, indicating a facultative xenogamous breeding system in *S. occidentalis*.

**Pollen viability and germination potential varied significantly among different sets of stamens** – Pollen viability and germination rate are two key aspects of pollen biology that play a key role in their ability to complete

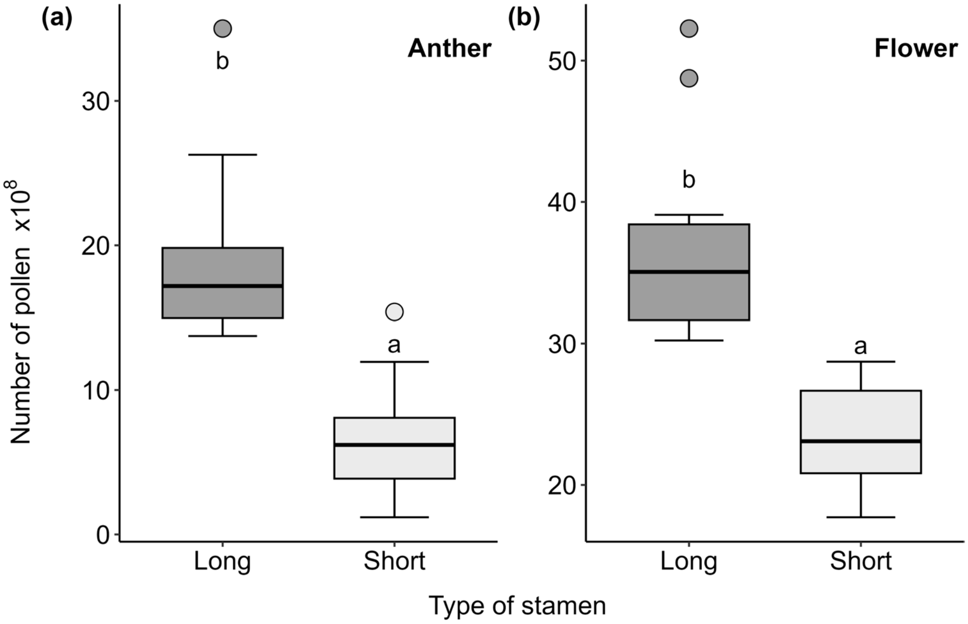

**Fig. 3** Median and percentiles (25–75%) of the **a** number of pollen per anther, and **b** flower from long and short stamens of *S. occidentalis* (N = 20 buds). Statistical analysis: number of pollen per anther (GLM: F = 136.5, $p < 0.001$) and per flower (GLM: F = 26.33, $p < 0.001$; Gaussian distribution). Different letters represented significant differences at a $p$-value < 0.05

post-pollination events (e.g., pollen tube growth and fertilisation of ovules). Pollen viability was assessed in three different periods of floral anthesis, viz., at bud stage, first day of flowering, and second day of flowering (i.e. flowers beginning to wilt) (Supplementary Table S1), using two different staining solutions, namely, acetocarmine and aniline blue. Between two staining treatments, the percentage viability of pollen grains that were produced by different sets of stamens of *S. occidentalis* was statistically significant (Fig. 4a) (GLM: *df* = 1, F value = 290.760, *p*-value < 0.0001). Further, we also observed statistically significant differences in pollen viability among different sets of stamens (GLM: *df* = 1, F value = 48.676, *p*-value < 0.0001), which suggested that the long-pollinating stamen produced pollen grains having higher fecundity than the short-pollinating stamen (Supplementary Table S1; Table 2). Pollen viability varied significantly among different periods of floral longevity (GLM: *df* = 2, F value = 156.767, *p*-value < 0.001). Additionally, the pollen germination percentage exhibited significant differences between different sets of stamens (GLM: *df* = 1, F value = 14.84, *p*-value < 0.001). The germination percentage was better in the long stamens (62.10%) than the short ones (55.47%) (Fig. 4b). Stigma receptivity was at its peak

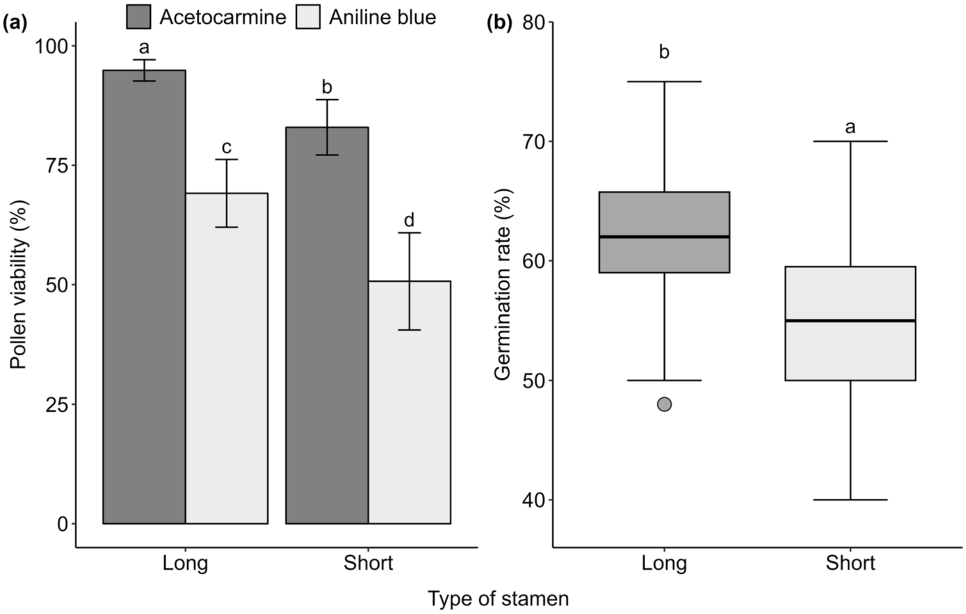

**Fig. 4** Pollen viability and germination percentage of pollen grains produced by different sets of stamens of *S. occidentalis*. **a** Pollen viability under two different staining solutions (acetocarmine and aniline blue; GLM: F value = 290.76, *p*-value < 0.001; Gaussian distribution), and **b** germination percentage (%) of pollens (GLM: F = 14.81, $p < 0.001$; Gaussian distribution). Different letters represented significant differences at a *p*-value < 0.05

**Table 2** Generalized linear model analysis with the effect of different stains, floral longevity (days) and types of stamens on pollen viability (log transformed) in *S. occidentalis*

| Model parameters | *df* | Mean Sq | F value | *p*-value |
|---|---|---|---|---|
| Stain | 1 | 8.902 | 290.760 | < 0.0001*** |
| Days | 2 | 4.800 | 156.767 | < 0.0001*** |
| Stamen | 1 | 1.490 | 48.676 | < 0.0001*** |
| Stain × Days | 2 | 2.292 | 74.844 | < 0.0001*** |
| Stain × Stamen | 3 | 0.202 | 6.605 | 0.0106* |
| Days × Stamen | 2 | 0.803 | 26.213 | 0.0001*** |
| Stain × Days × Stamen | 6 | 0.455 | 14.863 | < 0.0001*** |
| Residuals | 348 | 0.031 | | |

*p*-values at < 0.05 are in bold. The asterisks in the last column indicated a level of significance at different *p*-value: '***' < 0.001, '**' < 0.01, '*' < 0.05

during the first-day of flowering, and it remained receptive from anthesis to wilting.

***Senna occicentalis* showed a partially self-compatible breeding system, and pollen from both sets of stamens could be useful in its reproductive success** – Reproductive success in *S. occidentalis* was assessed using two parameters, viz., percent fruit set and mean seed set per fruit. In general, the fruit set (%), seed per fruit, and flower were significantly higher in hand-cross-pollinated flowers than in self-pollinated and bagged flowers for the fruit set (GLM: *df* = 7, F value = 13.35, *p*-value < 0.0001) and seed per fruit (GLM: *df* = 7, F value = 15.44, *p*-value < 0.0001), respectively (Table 3). The emasculated and bagged flowers did not produce any fruit or seed, which potentially indicated the absence of apomixis in *S. occidentalis*. Lower fruit and seed sets in autogamy and geitonogamy (applied self-pollination) -treated flowers suggested a partial self-compatible breeding system and dependency on animal pollinators for successful reproduction. However, the species mostly preferred cross-pollination by animal pollinators for increased reproductive success. The percentage of fruit set and seed per fruit in the supplementary hand-pollinated flowers were significantly higher than those in the naturally pollinated flowers (Table 3), which indicated that the species was pollen-limited in a natural environment. Fruit set and seed per fruit in hand cross-pollinated flowers using pollen grains from long stamens (fruit set: 24.78% and seed per fruit: 51.33 ± 7.00) were relatively higher compared to short stamens (fruit set: 15.96%, seed per fruit: 39.80 ± 12.13). Further, we observed significant differences in the percent fruit set (GLM: *df* = 1, F value = 6.251, *p*-value = 0.023) and mean seed per fruit (GLM: *df* = 1, F value = 4.255, *p*-value = 0.048) in inter-cross-pollination using long and short stamens (Table 3). Overall, the presence of fruit and seed sets in inter-cross-pollination treatments indicated that pollen from both sets of stamens contributed to the reproductive success of this species if effectively transferred by a pollen vector onto a receptive stigma.

**The large carpenter bees could be the most effective pollinators among the nine floral visitors of *S. occidentalis*** – Regarding pollination effectiveness (based on stigma touch frequency), three morphospecies were classified as "pollinators", and five morphospecies as "visitors" (Fig. 5 and Table 4). In the present study, a total of 204 interactions with floral visitors were recorded, representing three insect orders (Hymenoptera, Diptera, and Lepidoptera). The flowers were visited by nine species, which included three bees (*Xylocopa latipes, Xylocopa pubescence*, and *Lasioglossum* sp.), one ant (*Camponotus* sp.), and two wasps (*Xenorhynchium* sp., and an unidentified sp.) in the order Hymenoptera, one fly (*Mesembrius* sp.) in the order Diptera, and two butterflies (*Catopsilia pomona, Appias olferna*) in the order Lepidoptera. Insects from the Hymenoptera order represented 93.63% of all visits, followed by Lepidoptera (4.41%) and Diptera (1.96%). While collecting pollen from short-feeding

**Table 3** Details on breeding system of *S. occidentalis*

| Treatments | No of flowers | Percentage fruit set (%) | Mean seed per fruit |
|---|---|---|---|
| Autogamy | 234 (N = 16) | $5.45 \pm 9.44^{e}$ (n = 12) | $34.85 \pm 19.09^{de}$ |
| Apomixis | 56 (N = 16) | $0.00 \pm 0.00^{f}$ | $00.00 \pm 0.00^{e}$ |
| Emasculation (open) | 85 (N = 20) | $16.88 \pm 25.96^{c}$ (n = 13) | $45.20 \pm 13.77^{bcd}$ |
| Natural pollination | 395 (N = 20) | $27.31 \pm 7.30^{b}$ (n = 15) | $65.07 \pm 12.69^{bc}$ |
| Pollen supplement | 56 (N = 18) | $57.13 \pm 28.21^{a}$ (n = 32) | $70.39 \pm 12.24^{a}$ |
| Geitonogamy | 81 (N = 20) | $11.75 \pm 12.76^{d}$ (n = 10) | $24.40 \pm 20.16^{cde}$ |
| Hand cross-pollination | | | |
| Long Stamen | 76 (N = 18) | $24.78 \pm 21.45^{b}$ (n = 17) | $51.33 \pm 7.00^{b}$ |
| Short Stamen | 80 (N = 20) | $15.96 \pm 18.00^{c}$ (n = 14) | $39.80 \pm 12.13^{bcd}$ |

Different letters [a, b, c, d, e, f] in the last two column represented significant differences among treatments at *p*-value < 0.05 based on Tukey's post hoc test

N, number of individual plants (sample size); n, number of fruits

**Fig. 5** Dominant floral visitors of *S. occidentalis*. **a** *Xylocopa pubescens*, **b** *Xylocopa latipes* sitting on flowers; and **c** *Xylocopa pubescens,* and **d** *Xylocopa latipes* hovering around the flowers of *S. occidentalis*

**Table 4** Details of floral visitors, foraging hours, visit behaviour and visitation frequency in the natural population of *S. occidentalis*

| Order | Family | Name of taxa | Foraging hours | Nature of visit | Stigma touch | Visitation frequency/h |
|---|---|---|---|---|---|---|
| Diptera | Syrphidae | *Mesembrius* sp. | 11:00 a.m.–12:30 p.m. | Visitors/ Pollinators | + | $1.77 \pm 1.40^{bc}$ |
| Hymenoptera | Apidae | *Xylocopa latipes* | 08:00 a.m.–01:00 p.m. | Pollinators | +++ | $3.39 \pm 1.94^{ab}$ |
| | Apidae | *Xylocopa pubescens* | 08:00 a.m.–01:00 p.m. | Pollinators | +++ | $4.27 \pm 2.32^{a}$ |
| | Halictidae | *Lasioglossum* sp. | 09:30 a.m.–12:00 p.m. | Pollinators | ++ | $2.44 \pm 1.53^{bc}$ |
| | Formicidae | *Camponotus* sp. | 07:00 a.m.–02:00 p.m. | Visitors | – | $1.04 \pm 0.87^{c}$ |
| | Vespidae | *Xenorhynchium* sp. | 12:00 p.m.–02:00 p.m. | Visitors | – | $0.69 \pm 0.78^{c}$ |
| | Vespidae | Unidentified sp. | 12:00 p.m.–02:00 p.m. | Visitors | – | $0.87 \pm 0.93^{c}$ |
| Lepidoptera | Pieridae | *Catopsilia pomona* | 10:00 a.m.–11:00 a.m. | Visitors | ± | $0.92 \pm 1.09^{c}$ |
| | Pieridae | *Appias olferna* | 10:00 a.m.–11:30 a.m. | Visitors | ± | $1.29 \pm 1.21^{c}$ |

Different letters [a, b, c] in the last column indicated significant differences at *p*-value < 0.05 based on the Tukey's post hoc analysis

+++, excellent; ++, good; +, poor; ±, rarely; –, no touch to the stigma

stamens, the ventral body part of large carpenter bees (*Xylocopa* sp.) touched the stigma and long stamens. The pores of long stamens directed the pollen towards the lateral and ventral thorax of the bee, which was in direct contact with the stigma. On the other side, the short-feeding stamens directed pollen grains to the ventral and abdomen of bees. In contrast, the small-sized bees (*Lassioglossum* sp.) vibrated on long and short stamens separately by placing their belly on the apical pores of anthers and also came into contact with the stigma during pollen collection. However, butterfly species visited flowers to collect extra floral nectar and did not touch the stigma or anthers. Thus, they did not take part in pollen transfer.

– The visitation frequency (no. of visits/flower/h) values were the highest for *Xylocopa pubescence* (4.27 ± 2.32), followed by *Xylocopa latipes* (3.39 ± 1.94) and *Lasioglossum* sp. (2.44 ± 1.53). However, *Catopsilia pomona* (0.92 ± 1.09), an unidentified sp. (0.87 ± 0.93), and *Xenorhynchium* sp. (0.69 ± 0.78) showed a lower visitation frequency (Table 4). The combined differences in visitation frequencies were significant for floral visitors (GLM: $df = 8$, F = 2.47, $p$-value = 0.022). The visitation frequency of pollinators was significantly correlated with the number of open flowers (GLM: $df = 1$, F value = 13.75, $p$-value < 0.0001). Additionally, the visitation rate of large carpenter bees was significantly different among the stamen removal treatments (GLM: $df = 4$, F = 13.11, $p$-value < 0.0001). Compared to the intact flowers (controls), the flowers lacking long and short stamens received significantly lower visits (Fig. 6). Further, the removal of short stamens revealed a significant decrease (> 50%) in the visitation frequency compared to control flowers. This reduction was even higher than in long stamen-excluded flowers, suggesting a crucial role for the short stamens in a division of labour. However, the flowers devoid of all stamens received the least number of visits compared to the flowers with other stamen removal treatments. Further, the visitation choices of bees were not affected by the number of flowers observed per plant (GLM: $df = 1$, F value = 3.92, $p$-value = 0.51) or bee species (GLM: $df = 1$, F value = 0.42, $p$-value = 0.52).

## 4 Discussion

Flowers of *Senna occidentalis* exhibited ‘anther dimorphism’ and differential allotment of pollen resources to the long-pollinating and short-feeding stamens. These differential provisions of pollen quantity and quality with pollinator visitation behaviours among different sets of stamens in *S. occidentalis* corroborated the ‘division-of-labour’ hypothesis that was initially proposed by Müller (1881) and Darwin (1887). This momentous hypothesis predicted that the long stamens had pollen grains that were designed for pollination, while the pollen grains of the short stamens were for the nourishment of the pollinators (Müller 1883; Darwin 1887; Barrett 2010; Amorim et al. 2017). We observed significant variations in the quantity and fecundity of pollen from short-feeding stamens and long-pollinating stamens of *S. occidentalis*. The short-feeding stamens produced relatively larger pollen than the long-pollinating stamens. Moreover, the production of numerous smaller-sized pollen grains by long-pollinating stamens could be advantageous for pollination. Thus, current findings suggested a differential distribution of pollen grains in the feeding and pollinating stamens, supporting the division-of-labour hypothesis, especially in pollen-rewarding heterantherous flowers. However, further studies would be required to elucidate the genetic and evolutionary mechanisms for the division-of-labour within this large genus. In *S. occidentalis*, about 32% of the total pollen grains produced by each flower were available to pollinators in the feeding stamens. The high proportion of pollen availablity to pollinators has also been reported in other species of *Senna*, such as *S. reniformis* (G.Don) H.S.Irwin & Barneby (Mesquita-Neto et al. 2017), *S. pendula* (Humb. & Bonpl. Ex Willd.) H.S.Irwin & Barneby (Pinheiro-Costa et al. 2018), *S. bicapsularis* (L.) Roxb., and *S. alata* (L.) Roxb. (Luo et al. 2009). The availability of greater food volume might satisfy pollen-collecting bees, while the presence of a high number of viable pollen grains in pollinating stamens was crucial for the successful reproduction of the plant (Amorim et al. 2017; Mesquita-Neto et al. 2017). There were no significant differences in the size and volume of single pollen grains, which were produced by short and long stamens. However, the single long-pollinating stamen produced a higher number and volume of pollen grains than that of a short-feeding stamen. Also, the pollen grains of long-pollinating stamens exhibited higher pollen viability and germination percentages compared to the short-feeding stamens. Such a higher quantity and fecundity of pollen grains were also found in other members of *Senna,* namely *S. alata* (Luo et al. 2009), *S. bicapsularis* (Luo et al. 2009), *S. pendula* (Pinheiro-Costa et al. 2018), and *S. reniformis* (Mesquita-Neto et al. 2017).

Anther dimorphism has been evident in *S. occidentalis*, which is adopted by most of the nectarless insect-pollinated flowering plants that provide only pollen as a reward to their pollinators. Plants have evolved this trait, not only to compensate for the lack of reward to their pollinators but also to utilise pollen grains efficiently for pollination by insects that consume pollen (Vallejo-Marín et al. 2009; Barrett 2010). The species had a relatively lower pollen and pollen-to-ovule ratio, indicating this plant could have a facultative xenogamous breeding system (Cruden 1997). The flowers that offered only pollen as a prize to their pollinators were expected to generate additional pollen compared to the flowers that offered nectar or several incentives (Vogel

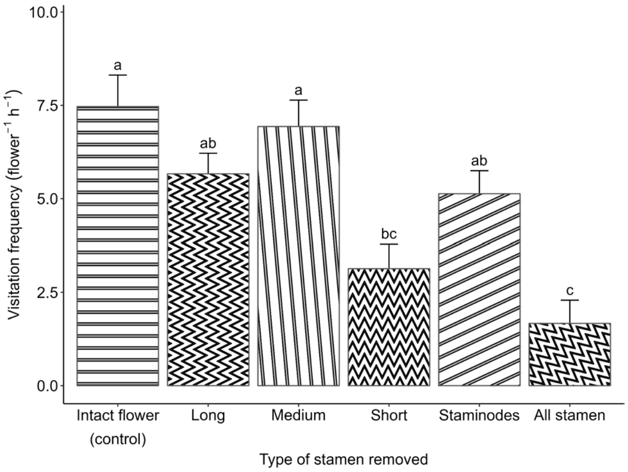

**Fig. 6** Floral visitation frequency under control (intact flower) and different stamen exclusion treatments in *S. occidentalis*. Statistical analysis: GLM F = 13.11, $p$-value < 0.0001; Gaussian distribution. Different letters represented significant differences at a $p$-value < 0.05

1978; Pinheiro-Costa et al. 2018). The lesser pollen generation than usual in *S. occidentalis* could be an evolutionary response to greater pollination efficiency, which was associated with pollen economy mediated by anther dimorphism (Ferreira and Araújo 2016).

Anther functional specialisation might be accompanied by differences in pollen traits such as viability and germination (Buchmann 1983; Vallejo-Marín et al. 2009). Both pollen viability and germination tests exhibited that the long-pollinating stamens of *S. occidentalis* generated pollen with a higher fecundity than that of short stamens. On the other side, the short-feeding stamens also produced viable pollen grains that were available for pollination by small buzzing bees (Hrycan and Davis 2005; Luo et al. 2009; Pinheiro-Costa et al. 2018). Several studies have reported that short stamens produced pollen grains that were either non-viable or had significantly lower viability than the pollination stamens (Decraene and Smets 2001; Veena and Nampy 2020). A higher proportion of pollen viability in pollinating stamens than in feeding stamens has been reported in many species of the genus *Senna*, including *S. alata*, *S. bicapsularis*, *S. siamea* (Lam.) H.S.Irwin & Barneby, and *S. reniformis* (Luo et al. 2009; Mesquita-Neto et al. 2017). These studies strongly suggested that the pollen of feeding stamens was entirely ineffective for reproductive success (e.g., fruit set and seed set) or was inferior to the pollen from long-pollinating stamens. However, in the present study, the pollen from the feeding stamens exhibited viability and germination potential, which indicated that these pollen grains could be available for pollination and successful fertilisation in *S. occidentalis*.

The presence of fruit and seed sets in autogamous self-pollination indicated the self-compatible breeding system in *S. occidentalis.* However, it exhibited dependency on pollen vectors for increased reproductive success. Previously, self-compatibility has been recorded in several members of the subtribe Cassiinae, including *S. multijuga* (Rich.) H.S.Irwin & Barneby, *Chamaecrista ramosa* (Vogel) H.S.Irwin & Barneby, and *S. silvestris* (Vell.) H.S.Irwin & Barneby (Carvalho and Oliveira 2003; Almeida et al. 2013). However, the genus *Senna* also had several species that exhibited self-incompatible breeding systems, namely *S. pendula*, *S. spectabilis* (DC.) H.S.Irwin & Barneby, and *S. martiana* (Benth.) H.S.Irwin & Barneby (Leite and Machado 2010; Pinheiro-Costa et al. 2018). The long stamens produced a higher proportion of fruit and seed sets in inter-cross-pollination between the long and short stamens. The current findings were in congruence with the previous studies revealing greater pollen fecundity in the long stamens compared to the other sets of stamens (Mesquita-Neto et al. 2017; Pinheiro-Costa et al. 2018).

The visitation frequency of effective pollinators has been strongly correlated with body size, and large bees had a high frequency of stigma contact (Mesquita-Neto et al. 2021). Consequently, a floral visitor was more likely to touch the anther and stigma when their body dimension corresponded to and/or exceeded the anther-stigma distance. In *S. occidentalis*, the large carpenter bees touched the stigma more frequently compared to the small bees (*Lassioglossum* sp.) and flies (*Mesembrius* sp.). These large bees carried conspecific pollen on their body sites that touched the stigma, and therefore, even a few visits might lead to the deposition of an adequate number of fertile pollen on the stigma. In the current study, the test flowers, with the removal of all sets of stamens, showed a significantly lower frequency of visits from *Xylocopa* sp. Further, the removal of short stamens indicated a significantly lower visitation frequency of these pollinators compared to long stamens removed and control flowers. However, these flowers were able to set fruit and seeds, which indicated the effectiveness of these bees. Thus, these findings revealed that the short stamens might play a vital role in *S. occidentalis* not only in attracting pollinators but also in increased reproductive success (fruit and seed set) by performing duel roles as feeding as well as pollinating functions (especially by small-sized bees). Similarly, previous studies have also shown that the small and medium-sized bees visited indiscriminately to stamen removal experimented flowers and collected pollen from both feeding and pollinating stamens and subsequently contributed to pollination and reproduction (Vallejo-Marín et al. 2010; Mesquita-Neto et al. 2017; Papaj et al. 2017). The division of labour did not apply to the small-sized bees because they did not show a specific body positioning during flower handling as shown by the large bees (*Xylocopa* sp.). The small bees collected pollen from the flower surface and buzzed indiscriminately on single anthers to access the pollen contents (Solís-Montero et al. 2015; Mesquita-Neto et al. 2021). However, earlier researchers have shown that small floral visitors were poor pollinators, especially for the buzz-pollinated flowers with poricidal anthers (Rego et al. 2018; Konzmann et al. 2020). Thus, the body size of floral visitors and the size of flowers could be considered as an important predictors of pollination efficiency in buzz-pollinated plant species (De Luca et al. 2019; Mesquita-Neto et al. 2021).

The buzz-pollinated, nectarless flowers provided only pollen as a reward to their pollinators, which might impose a selection pressure to retain the pollinator of interest. This was experimentally demonstrated by comparing the foraging behaviours of pollinators on the test (flowers with anthers removed) and control (unmanipulated) flowers. Our findings from stamen exclusion experiments demonstrated a potential division of labour as the short stamens could contribute mostly to feed their pollinators, while the large stamens were responsible for pollination and reproductive success. Besides, the floral visitation frequency was significantly reduced in the test flowers compared to unmanipulated control flowers, and large carpenter bees rarely buzzed on stamen-removed flowers for a second time. These findings were in congruence with previous studies showing that these bees accessed pollen during sonication (Luo et al. 2008; Mesquita-Neto et al. 2017). The visitation frequency was significantly reduced in flowers with no short feeding stamens, which demonstrated that their bright yellow colour might attract the large carpenter bees (Lou et al. 2008). However, several studies have also reported that small-sized bees equally visited flowers with only one set of stamens, either pollinating or feeding stamens (Luo et al. 2008; Mesquita-Neto et al. 2017). Moreover, some buzz-pollinated flowers might suffer loss of reproductive output due to pollen theft, especially from the small bees that could buzz only individual anthers, and in most instances, they did not come in contact with the stigmas (Renner 1983). In many instances, *Trigona* bees pierce or chew the poricidal anthers of buzz-pollinated flowers in search of pollen, which might no longer be available to the *Xylocopa* bees to transfer and deposit the pollen onto the stigma (Rego et al. 2018; Telles et al. 2019). The observed differences in sonicating preferences of large bees (*Xylocopa* sp.) in this study could support the tenets of the division-of-labour hypothesis that the pollinators preferred collecting pollen from one type of stamen, i.e., the pollinating stamens (Vallejo-Marín et al. 2009; Papaj et al. 2017). Earlier studies have also revealed that pollinators preferred to sonicate (collect pollen from) short-feeding stamens than long-pollinating stamens (Amorim et al. 2017; Papaj et al. 2017; Saab et al. 2021). Overall, these observations indicated that bees (*Xylocopa* sp.) might be attracted to the bright yellow structures (i.e., stamens) in flowers and could be deceived by this signal regardless of the species (Lunau 2000; Hegland and Totland 2005; Velloso et al. 2018).

In summary, the flowers of *S. occidentalis* possessed two kinds of stamens, long and short, which were often assumed to perform pollinating and feeding functions, respectively. The short-feeding stamens produced pollen with low quality and quantity. Subsequently, they could be recognised as the visual signal for effective pollinators, indicating the presence of floral resources. The long stamens were related to pollination function and deposited pollen on the safer sites on the body of effective pollinators for efficient transfer and successful fertilisation. Breeding system experiments suggested that the species was pollen-limited and dependent on pollen vectors for reproductive success. Total nine floral visitors foraged on the flowers of *S. occidentalis*. Among them, the large carpenter bees (*X. latipes* and *X. pubescence*) could be considered the most effective pollinators. The number, position, and symmetry of stamens varied greatly in the genus *Senna*. Thus, present findings of differential distributions of pollen quantity, quality, and pollinator visitation behaviours on different sets of stamens substantiated the division-of-labour hypothesis in *S. occidentalis*.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s40415-024-01032-9.

**Acknowledgements** Authors thank the Heads, Dept. of Botany, MBB and other authorities of Tripura University for providing necessary facilities to carry out this study. SP thanks University Grant Commission (UGC), New Delhi, India for providing non-NET PhD fellowship and is grateful to Dr. Joydeb Majumder (Dept. of Zoology, Tripura University, Tripura, India) and Mr. Arnob Chakrovorty (Dept. of Zoology, University of Kalyani, West Bengal, India), for their assistance during insect identification.

**Author contributions** SP, BBD and BKD designed the plan of work. SP and BG carried out the fieldwork and collected data. SP carried out the statistical analysis and prepared the initial draft of the manuscript, main/ supplementary figures and tables with BG and BBD. SP, BKD and BBD edited the revised drafts and finalized the manuscript. Subsequently, all authors have read and approved the final version of the manuscript.

**Data availability** The data used to support the conclusions of this article is included within the article or in the online supplementary files.

## Declarations

**Conflict of interest** All authors declare that there are no financial/commercial conflicts of interest.

## References

Almeida NM, Castro CC, Leite AVL, Novo RR, Machado IC (2013) Enantiostyly in *Chamaecrista ramose* (Fabaceae-Caesalpinioideae): floral morphology, pollen transfer dynamics and breeding system. Plant Biol 15:369–375. https://doi.org/10.1111/j.1438-8677.2012.00651.x

Amorim T, Marazzi B, Soares AA, Forni-Martins ER, Muniz CR, Westerkamp C (2017) Ricochet pollination in *Senna* (Fabaceae)–petals deflect pollen jets and promote division of labour among flower structures. Plant Biol 19:951–962. https://doi.org/10.1111/plb.12607

Barrett SC (2010) Darwin's legacy: the forms, function and sexual diversity of flowers. Philos Trans R Soc Lond B Biol Sci 365:351–368

Basso-Alves JP, Goldenberg R, Teixeira SP (2022) Connective modifications and origin of stamen diversity in Melastomataceae. J Plant Res 135:659–680. https://doi.org/10.1007/s10265-022-01405-9

Bionet-Eafrinet (2016) Invasive plants key and fact sheets. http://keys.lucidcentral.org/keys/v3/eafrinet/index.htm

Brewbaker JL, Kwack BH (1963) The essential role of calcium ion in pollen germination and pollen tube growth. Am J Bot 50:859–865

Buchmann SL (1974) Buzz pollination of *Cassia quiedondilla* (Leguminosae) by bees of the genera *Centris* and *Melipona*. Bull South Calif Acad Sci 73:171–173

Buchmann SL (1983) Buzz pollination in angiosperms. In: Jones CE, Little RJ (eds) Handbook of experimental pollination biology. Van Nostrand Reinhold, New York, pp 73–133

Carvalho DA, Oliveira PE (2003) Biologia reprodutiva e polinização de *Senna sylvestris* (Vell.) H.S. Irwin & Barneby (Leguminosae, Caesalpinioideae). Rev Bras Bot 26:319–328

Chen JC, Fang SC (2016) The long pollen tube journey and in vitro pollen germination of *Phalaenopsis* orchids. Plant Reprod 29:179–188. https://doi.org/10.1007/s00497-016-0280-z

Cruden RW (1977) Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. Evol 31:32–46. https://doi.org/10.1111/j.15585646.1977.tb00979.x

Dafni A, Firmage D (2000) Pollen viability and longevity: practical, ecological and evolutionary implications. Plant Syst Evol 222:113–132

Darwin C (1887) The life and letters of Charles Darwin including an autobiographical chapter. In: F. Darwin (ed), vol 3, John Murray, London

De Luca PA, Vallejo-Marín M (2013) What's the 'buzz' about? The ecology and evolutionary significance of buzz-pollination. Curr Opin Plant Biol 16:429–435. https://doi.org/10.1016/j.pbi.2013.05.002

De Luca PA, Buchmann S, Galen C, Mason AC, Vallejo-Marín M (2019) Does body size predict the buzz-pollination frequencies used by bees? Ecol Evol 9:4875–4887. https://doi.org/10.1002/ece3.5092

Decraene LPR, Smets EF (2001) Staminodes: their morphological and evolutionary significance. Bot Rev 67:351–402

Dellinger AS, Pöllabauer L, Loreti M, Czurda J, Schönenberger J (2019) Testing functional hypotheses on poricidal anther dehiscence and heteranthery in buzz-pollinated flowers. Acta ZooBot Austria 156:197–214

Dulberger R (1981) The floral biology of *Cassia didymobotrya* and *C. auriculata* (Caesalpiniaceae). Am J Bot 68:1350–1360. https://doi.org/10.1002/j.1537-2197.1981.tb07846.x

Dulberger R, Smith MB, Bawa KS (1994) The stigmatic orifice in *Cassia*, *Senna*, and *Chamaecrista* (Caesalpiniaceae): morphological variation, function during pollination, and possible adaptive significance. Am J Bot 81:1390–1396. https://doi.org/10.1002/j.1537-2197.1994.tb15623.x

Endress PK (1996) Diversity and evolutionary trends in angiosperm anthers. In: D'Arcy G, Keating RC (eds) The anther: form, function and phylogeny, Cambridge University Press, Cambridge, pp 92–110

Ferreira QIX, Araújo FPD (2016) Pollen economy enhanced by heteranthery in *Desmocelis villosa* (Melastomataceae). Rodriguésia 67:347–355. https://doi.org/10.1590/2175-7860201667206

Gottsberger G, Silberbauer-Gottsberger I (1988) Evolution of flower structures and pollination in Neotropical Cassiinae (Caesalpiniaceae) species. Phyton 28:293–320

Graham SW, Barrett SCH (1995) Phylogenetic systematics of Pontederiales: implications for breeding-system evolution. In: Rudall PJ, Cribb PJ, Cutler DF, Humphries CJ (eds) Monocotyledons: systematics and evolution. Royal Botanical Gardens, Kew, pp 415–441

Hegland SJ, Totland Q (2005) Relationships between species' floral traits and pollinator visitation in a temperate grassland. Oecologia 145:586–594. https://doi.org/10.1007/s00442-005-0165-6

Hrycan WC, Davis AR (2005) Comparative structure and pollen production of the stamens and pollinator-deceptive staminodes of *Commelina coelestis* and *C. Dianthifolia* (Commelinaceae). Ann Bot 95:1113–1130. https://doi.org/10.1093/aob/mci134

Irwin HS, Barneby RC (1981) Tribe 2: Cassieae Bronn (1822). In: Pohlhill RM, Raven PH (eds) Advances in legume systematics. Kew, Royal Botanic Gardens, pp 97–106

Kay KM, Jogesh T, Tataru D, Akiba S (2020) Darwin's vexing contrivance: a new hypothesis for why some flowers have two kinds of anther. Proc R Soc B 287:20202593. https://doi.org/10.1098/rspb.2020.2593

Kearns CA, Inouye DW (1993) Techniques for Pollination Biologists. University Press of Colorado, Niwot, CO, pp 77–151

Khatun S, Flowers TJ (1995) The estimation of pollen viability in rice. J Exp Bot 46:151–154. https://doi.org/10.1093/jxb/46.1.151

Konzmann S, Hilgendorf F, Niester C, Lunau K (2020) Morphological specialisation of heterantherous Rhynchanthera grandiflora (Melastomataceae) accommodates pollinator diversity. Plant Biol 22:583–590. https://doi.org/10.1111/plb.13102

Lazaro A, Jakobsson A, Totland Q (2013) How do pollinator visitation rate and seed set relate to species' floral traits and community context? Oecologia 173:881–893. https://doi.org/10.1007/s00442-013-2652-5

Leite AV, Machado IC (2010) Reproductive biology of woody species in Caatinga, a dry forest of north-eastern Brazil. J Arid Environ 74:1374–1380. https://doi.org/10.1016/j.jaridenv.2010.05.029

Liu H, Pemberton R (2009) Solitary invasive orchid bee outperforms co-occurring native bees to promote fruit set of an invasive *Solanum*. Oecologia 159:515–525. https://doi.org/10.1007/s00442-008-1232-6

Lum Nde A, Chukwuma CI, Erukainure O, Chukwuma M, Matasbisa M (2021) Ethnobotanical, phytochemical, toxicology and anti-diabetic potential of *Senna occidentalis* (L.) link: A review. J Ethnopharmacol 283:114663. https://doi.org/10.1016/j.jep.2021.114663

Lunau TDK (2000) The ecology and evolution of visual pollen signals. Plant Syst Evol 222:89–111

Lunau K, Piorek V, Krohn O, Pacini E (2015) Just spines mechanical defense of malvaceous pollen against collection by corbiculate bees. Apidologie 46:144–149. https://doi.org/10.1007/s13592-014-0310-5

Luo Z, Zhang D, Renner SS (2008) Why two kinds of stamens in buzz-pollinated flowers? Experimental support for Darwin's division-of-labour hypothesis. Funct Ecol 22:794–800. https://doi.org/10.1111/j.1365-2435.2008.01444.x

Luo Z-L, Gu L, Zhang D-X (2009) Intrafloral differentiation of stamens in heterantherous flowers. J Syst Evol 47:43–56. https://doi.org/10.1111/j.1759-6831.2009.00002.x

Marazzi B, Endress PK (2008) Patterns and development of floral asymmetry in *Senna* (Leguminosae, Cassiinae). Am J Bot 95:22–40. https://doi.org/10.3732/ajb.95.1.22

Mesquita-Neto JN, Costa BKP, Schlindwein C (2017) Heteranthery as a solution to the demand for pollen as food and for pollination: legitimate flower visitors reject flowers without feeding anthers. Plant Biol 19:942–950. https://doi.org/10.1111/plb.12609

Mesquita-Neto JN, Blüthgen N, Schlindwein C (2018) Flowers with poricidal anthers and their complex interaction networks: disentangling legitimate pollinators and illegitimate visitors. Funct Ecol 32:2321–2332. https://doi.org/10.1111/1365-2435.13204

Mesquita-Neto JN, Vieira ALC, Schlindwein C (2021) Minimum size threshold of visiting bees of a buzz-pollinated plant species: consequences for pollination efficiency. Am J Bot 108:1006–1015. https://doi.org/10.1002/ajb2.1681

Michener CD (1962) An interesting method of pollen collecting by bees from flowers with tubular anthers. Rev Biol Trop 10:167–175

Mishra P, Pandey CM, Singh U, Gupta A, Sahu C, Keshri A (2019) Descriptive statistics and normality tests for statistical data. Ann Card Anaesth 22(1):67–72. https://doi.org/10.4103/aca.ACA_157_18

Müller H (1881) Two kinds of stamens with different functions in the same flower. Nature 24:307–308

Müller F (1883) Two kinds of stamens with different functions in the same flower. Nature 27:364–365

Ne´eman G, Ju¨rgens A, Newstrom-Lloyd L, Potts SG, Dafni A (2009) A framework for comparing pollinator performance: effectiveness and efficiency. Biol Rev 85:435–451. https://doi.org/10.1111/j.1469-185X.2009.00108.x

Nogueira A, Valadão-Mendes LB, El Ottra JH, Guimarães E, Cardoso-Gustavson P, Quinalha MM, Paulino JV, Rando JG (2018) Relationship of floral morphology and development with the pattern of bee visitation in a species with pollen-flowers, *Chamaecrista desvauxii* (Fabaceae). Bot J Linn Soc 187:137–156. https://doi.org/10.1093/botlinnean/boy008

Oviedo Prieto R, Herrera Oliver P, Caluff MG et al (2012) National list of invasive and potentially invasive plants in the Republic of Cuba–2011. Bissea 6:22–96

Owens SJ, Lewis GP (1989) Taxonomic and functional implications of stigma morphology in species of *Cassia, Chamaecrista,* and *Senna* (Leguminosae: Caesalpinioideae). Plant Syst and Evol 163:93–105. https://doi.org/10.1007/BF00936157

Papaj DR, Buchmann SL, Russell AL (2017) Division of labor of anthers in heterantherous plants: flexibility of bee pollen collection behavior may serve to keep plants honest. Arthropod Plant Interact 11:307–315. https://doi.org/10.1007/s11829-017-9497-5

Paul S, Dholakia BB, Datta BK (2024) Reproductive biology and pollination ecology of *Ipomoea triloba* L. (Convolvulaceae): an alien invasive species of the Indo-Burma biodiversity hotspot. Plant Spec Biol 39:61–76. https://doi.org/10.1111/1442-1984.12443

Paulino JV, de Freitas MV, Prenner G (2016) Evidence for division of labor and division of function related to the pollen release in Papilionoideae (Leguminosae) with a heteromorphic androecium. Int J Plant Sci 177:590–607. https://doi.org/10.1086/687351

Pier (2016) Pacific Islands ecosystems at risk. Honolulu: HEAR, University of Hawaii, USA

Pinheiro-Costa BK, Mesquita-Neto JN, Rego JO, Schlindwein C (2018) Trade off between quantity and size of pollen grains in the heterandrous flowers of *Senna pendula* (Fabaceae). Acta Bot Bras 32:446–453

Prota (2016) PROTA4U web database. Wageningen, Netherlands: Plant resources of tropical Africa, Wageningen and Nairobi, Kenya Natherlands\Nairobi

R Core Team (2022) R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rego JO, Oliveira R, Jacobi CM, Schlindwein C (2018) Constant flower damage caused by a common stingless bee puts survival of a threatened buzz-pollinated species at risk. Apidologie 49:276–286. https://doi.org/10.1007/s13592-017-0552-0

Renner S (1983) The widespread occurrence of anther destruction by *Trigona* bees in Melastomataceae. Biotropica 15:251–256. https://doi.org/10.2307/2387649

Saab GD, Mansano VD, Nogueira A, Maia IC, Bergamo PJ, Paulino JV (2021) A sophisticated case of division of labour in the trimorphic stamens of the *Cassia fistula* (Leguminosae) flower. AoB Plants 13:054. https://doi.org/10.1093/aobpla/plab054

Shivanna KR, Tandon R (2014) Reproductive ecology of flowering plants: a manual. Springer, Heidelberg

Shivanna KR, Rangaswamy NS (1992) Pollen biology: a laboratory manual. Springer, Berlin/Heidelberg

Solís-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. Ecol Evol 7:2706–2715. https://doi.org/10.1002/ece3.2897

Solís-Montero L, Vergara CH, Vallejo-Marín M (2015) High incidence of pollen theft in natural populations of a buzz-pollinated plant. Arthropod-Plant Interact 9:599–611. https://doi.org/10.1007/s11829-015-9397-5

Solís-Montero L, Cáceres-García S, Alavez-Rosas D, García-Crisóstomo JF, Vega Polanco M, Grajales Conesa J, Cruz-López L (2018) Pollinator preferences for floral volatiles emitted by dimorphic anthers of a buzz pollinated herb. J Chem Ecol 44:1058–1067. https://doi.org/10.1007/s10886-018-1014-5

Telles FJ, Gonzálvez FG, Rodríguez-Gironés MA, Freitas L (2019) The effect of a flower-dwelling predator on a specialized pollination system. Biol J Linn Soc 126:521–532. https://doi.org/10.1093/biolinnean/bly184

Vallejo-Marín M, Manson JS, Thomson JD, Barrett SCH (2009) Division of labour within flowers: heteranthery, a floral strategy to reconcile contrasting pollen fates. J Syst Evol 22:828–839. https://doi.org/10.1111/j.1420-9101.2009.01693.x

Vallejo-Marín M, Silva EM, Sargent RD, Barrett SCH (2010) Trait correlates and functional significance of heteranthery in flowering plants. New Phytol 188:418–425. https://doi.org/10.1111/j.1469-8137.2010.03430.x

Vallejo-Marín M, Pereira Nunes CE, Russell AL (2022) Anther cones increase pollen release in buzz-pollinated *Solanum* flowers. Evolution 76:931–945. https://doi.org/10.1111/evo.14485

Veena V, Nampy S (2020) Heteromorphic stamen: a strategy in nectarless entomophilous plants to increase pollination efficiency? An investigation with regard to three species of Commelinaceae. Plant Syst Evol 306:82. https://doi.org/10.1007/s00606-020-01710-7

Velloso M, d Brito VLG, Catano APS, Romero R (2018) Anther specializationsrelated to the division of labor in M*icrolicia cordata* (Spreng.) Cham. (Melastomataceae). Acta Bot Bras 32:349–358. https://doi.org/10.1590/0102-33062017abb0358

Vivarelli D, Petanidou T, Nielsen A, Cristofolini G (2011) Small-size bees reduce male fitness of the flowers of *Ononis masquillierii* (Fabaceae), a rare endemic plant in the northern Apennines. Bot J Linn Soc 165:267–277. https://doi.org/10.1111/j.1095-8339.2010.01105.x

Vogel S (1978) Evolutionary shifts from reward to deception in pollen flowers. In: Richards AJ (ed) The pollination of flowers by insects. Linnean Society Symposium Series Number 6. Academic, London