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# Staminal variation and its possible significance in *Commelina benghalensis* L. and *Commelina caroliniana* Walter

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**Flowers of *Commelina benghalensis* L. and *Commelina caroliniana* Walter are odourless and nectar-less. Therefore, they rely on visual characteristics for attracting pollinators and offer pollen as the only reward. Androecium, the focus of an insect's attention in these species, consists of three fertile and three sterile stamens. The three fertile stamens have two types of anthers. The central one has a bright yellow, long, inwardly curved, versatile anther with a massive connective. The other two are lateral, cryptic, with smaller ovate, straight and basifixed anthers, light grey in *C. benghalensis* and yellow with a brown streak in *C. caroliniana*. The sterile stamens or staminodes are bright yellow, variously shaped and borne on slender filaments. They contrast sharply with the blue corolla and attract insects by appearing filled with abundant pollen. To determine the possible significance of this diversity, morphological and functional aspects of the male reproductive organs were worked out and, between and within species comparisons were also made.**

**Keywords:** Androecium, *Commelina benghalensis* L., *Commelina caroliniana* Walter, heteranthery, staminode.

*COMMELINA* L., a monocotyledonous genus belonging to the family Commelinaceae is represented by about 170 species[1]. The family as a whole is characterized by the production of relatively small, delicate, ephemeral and nectar-less flowers, which bloom for a brief period in a day[2–10].

Production of nectar-less flowers with brief opening time has a direct impact on the reproductive biology of *Commelina*[2,3]. Lack of nectar has two utmost implications, i.e. inability of the flowers to attract nectar-loving (nectarivorous) pollinators and compulsion of producing pollen in ample quantity that would suffice for pollination and for rewarding the pollinator[2,3]. Similarly, short blooming period of an individual flower limits temporal separation or sequential development of the male and female sexual organs or functions in the flower[2].

Therefore, the main source of attraction to insect visitors shall be visual characteristics of the flowers that include brightly coloured petals, showy fertile stamens and showy infertile staminodes[3], and even variously

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coloured floral axes, cincinni, bracts or peduncle[2]. Of these, the stamens whether fertile or vestigial, should be the focal point of an insect's attention as pollen is the only reward that these flowers offer.

*Commelina benghalensis* L. and *Commelina caroliniana* Walter are two prolific weeds which grow in abundance during the rainy season in tropical and subtropical regions of India. The two species are andromonoecious, i.e. bear male and bisexual flowers (Figure 1). While all the flowers of *C. caroliniana* are borne on aerial shoots and are uniformly chasmogamous, those of *C. benghalensis* are sexually diverse[11,12] and differentiate on three types of branches. These are: the aerial (negatively geotropic), the subterranean (positively geotropic) and sub-aerial branches[4–6,10]. All types of branches bear flowers encased in green spathes. Flowers are generally of three types, viz. male chasmogamous (male), bisexual chasmogamous (CH) and bisexual cleistogamous (CL). Plants of *C. caroliniana* also show variability in their floral structure and function[9]. Besides working out the reproductive biology of these two taxa, the specific objective of the present study was to compare the morphological and functional aspects of the male reproductive organs within and between species.

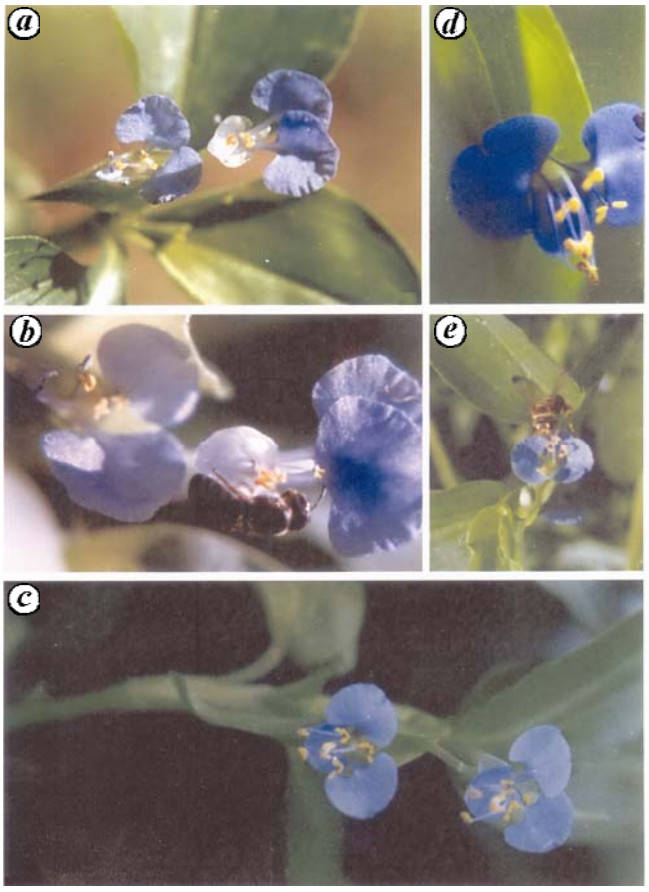


**Figure 1** ***a*–*e*.** *Commelina benghalensis* (***a***, ***b***; × 4.8) and *Commelina caroliniana* (***c*–*e***; × 2) flowers in bloom. Species of (***b***) *Halictus* and (***e***) *Steganomus* foraging flowers of the two species respectively.

Inflorescences ($N$ = 40 each) of *C. benghalensis* and *C. caroliniana* varying in age were collected from different populations growing naturally or those that have been naturalized in the Botanical Garden of the Department of Botany, University of Jammu (see refs 5, 6, 9 and 10 for details).

The fresh collections were fixed in Carnoy's fluid (three parts of absolute alcohol and one part of glacial acetic acid) for 6–8 h and then transferred to 70% ethanol till further use. Specimens were dissected under a dissection microscope to remove mature and immature flowers from the cyme. The staminodes from both were then excised with the help of sterile and sharp forceps. After washing with water, these were stained in Lewis'[13] stain and mounted in a drop of lacto-phenol on a fresh slide, examined microscopically and photographed.

The number of pollen grains per anther or antherode was estimated separately for the lateral and central stamens, and the staminodes according to the methodology given by Kaul *et al.*[5], and so was pollen viability. For this purpose, pollen grains from staminodes and freshly dehisced stamens were stained with 1% acetocarmine, aniline blue and Lewis' stain separately to determine the pollen viability.

The size of individual organs was measured using vernier calipers and that of pollen grains with calibrated ocular and stage micrometers.

In order to determine how far the central versatile anther of the chasmogamous flower contributes to wind pollination, hanging slide experiments were carried out. Glass slides smeared with Mayer's albumin (filtered egg albumin and glycerin in the ratio of 1 : 1 and a pinch of sodium salicylate or thymol) were hung from 35 cm high, T-shaped wooden stands fixed at a distance of 25–50 cm all around the individual plants growing in the experimental beds. The slides were screened microscopically and scored for pollen load following 24 h exposure.

The plants bloom in the morning (5.30 h). Six types of pollination treatments were undertaken to determine the possible role of staminodes and two types of stamens in the mating system:

(A) Mature flower buds ($n$ = 40) were emasculated 12 h before anthesis by removing the three fertile stamens and keeping the vestigial ones intact.

(I) One set was tagged and left undisturbed for pollination to take place as it does in nature. This was done to see whether insects get attracted by the sterile anthers and visit these flowers.

(II) The second set was bagged to test whether pollen of vestigial anthers can cause pollination on its own.

(B) Mature flower buds ($n$ = 80) were emasculated 12 h before anthesis by removing all staminodes and stamens along with their filaments.

(III) One set was then pollinated with pollen collected from central as well as lateral stamens of CH flowers of other spathes of the same branch.

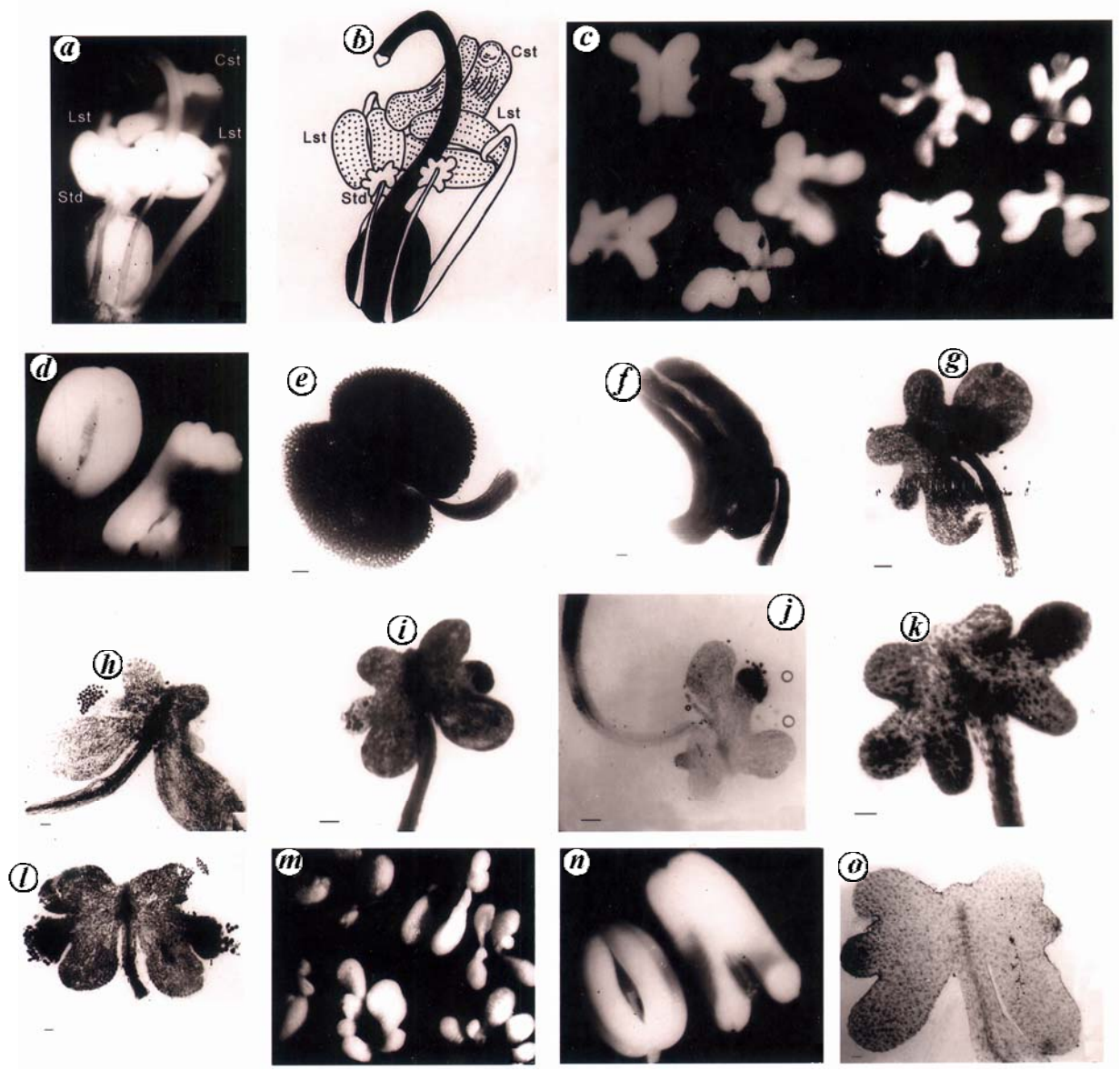


**Figure 2** ***a*–*o*.** Stereo (***a***, ***c***, ***d***, ***m***, ***n***) and light (***e***–***l***, ***o***) photomicrographs of fertile and sterile anthers of (***a***–***l***) *C. benghalensis* and (***m***–***o***) *C. caroliniana*. ***a***, Aerial chasmogamous flower of *C. benghalensis* with petals and sepals removed depicting the relative position of two types of fertile anthers (Lst, Lateral stamen; Cst, Central stamen), staminodes (Std) and stigma (× 22). ***b***, Explanatory drawing thereof. Note the third staminode is hidden partly by the ovary and partly by one of the lateral stamens. Anthers of staminodes of flowers of *C. benghalensis* (***c***, ***g***–***l***) and *C. caroliniana* (***m***, ***o***). Note the varied patterns of size, shape and lobing. ***i***, ***k***, ***l***, The two middle fertile lobes. ***g***, ***h***, ***j***, A single fertile lobe. ***o***, Lobes devoid of pollen. Anthers of lateral and central stamens of (***d***–***f***) subterranean (× 42) flower of *C. benghalensis* and (***n***) second-order flower (× 25) of *C. caroliniana*. (Anthers on the left and right belong to the lateral and central stamens respectively.) Bar = 30 μm.

(IV) The second set was pollinated with pollen collected from central as well as lateral stamens of CH flowers of plants belonging to a different population.

(V) The third set was bagged.

(VI) The fourth set was tagged and left undisturbed for pollination to take place as it does in nature.

Data on the number and behaviour of insect visitors to flowers were collected through visual observations made between 5 : 30 and 12 : 00 h on sunny days.

Data collected were analysed by applying two-way ANOVA to determine the following: (a) The effect of pollen type (stamen, staminode) and flower type/order on (i) filament length, (ii) anther length, (iii) pollen length and (iv) pollen breadth. (b) The effect of pollen type (lateral and central stamen, staminode) on pollen count after transforming it logarithmically[14,15].

Student's *t*-test was applied to test the significance of the difference[14,15] in the viability and size (length and breadth) of the pollen grains produced by the stamens and staminodes of *C. caroliniana*.

All photomicrography was undertaken on temporary slides using Kodalith orthofilm 6556, type-3 photographic film and Olympus PM6 35 mm camera. Stereo-

**Table 1.** Morphometry of the essential floral organs of the two species of *Commelina*

| Flower type/order | Filament length (mm) | | Anther length (mm) of stamen | | Pistil length (mm) |
|---|---|---|---|---|---|
| | Staminode | Stamen | Central | Lateral | |
| *Commelina benghalensis* | | | | | |
| Male | 3.7 ± 0.11* (2.5–5.0)** | 5.8 ± 0.98 (4.0–7.2) | 1.65 ± 0.01 (1.5–1.75) | 1.16 ± 0.014 (0.98–1.35) | – |
| A CH | 3.2 ± 0.05 (2.0–5.0) | 5.2 ± 0.08 (3.0–7.0) | 1.44 ± 0.026 (1.2–1.6) | 1.107 ± 0.013 (0.95–1.28) | 7.6 ± 0.12 (5.5–9.5) |
| A CL | 1.7 ± 0.04 (1.0–2.5) | 2.7 ± 0.05 (1.7–3.5) | 1.43 ± 0.015 (1.28–1.6) | 0.99 ± 0.011 (0.87–1.15) | 3.8 ± 0.06 (3.2–4.7) |
| SA | 3.1 ± 0.07 (2.5–4.0) | 4.7 ± 0.08 (4.0–6.0) | – | – | 6.0 ± 0.12 (5.5–7.0) |
| ST | 1.3 ± 0.12 (1.0–2.0) | 1.9 ± 0.04 (1.5–2.5) | 0.97 ± 0.035 (0.9–1.03) | 0.64 ± 0.01 (0.53–0.75) | 2.7 ± 0.08 (2.2–3.3) |
| *Commelina caroliniana* | | | | | |
| Male | 6.0 ± 0.1 (5.0–7.0) | 9.0 ± 0.2 (6–11) | 1.8 ± 0.03 (1.6–1.97) | 1.14 ± 0.01 (1.0–1.3) | – |
| 1 | 5.2 ± 0.08 (4.0–6.5) | 7.0 ± 0.1 (5–9) | 1.7 ± 0.03 (1.45–1.93) | 1.07 ± 0.01 (0.92–1.21) | 9.0 ± 0.1 (7.5–9.5) |
| 2 | 1.8 ± 0.25 (0.1–5) | 4.2 ± 0.04 (0.8–8.0) | 1.624 ± 0.04 (1.31–1.94) | 1.09 ± 0.021 (0.98–1.21) | 6.0 ± 0.7 (1.0–9.0) |
| 3 | 0.4 ± 0.07 (0.3–0.9) | 2.9 ± 0.05 (1.7–4.2) | 1.4 ± 0.06 (1.1–1.7) | 0.98 ± 0.05 (0.7–1.2) | 0.7 ± 0.06 (0.3–1.0) |
| 4 | – | – | 1.26 ± 0.09 (0.98–1.89) | 0.89 ± 0.06 (0.55–1.17) | – |

*Mean ± SE; **Range.

photomicrography was carried out from fresh specimens using Stereomicroscope MeOpta.

A spathe or folded bract encloses the inflorescences of the two species of *Commelina* (Figure 1). Within each spathe there are two cymes: a lower one with usually 2–3 buds in *C. benghalensis* and 4–5 in *C. caroliniana* and the upper cyme with mostly one bud in both the species. The lower cyme carries hermaphrodite flowers and upper a staminate flower[9,10].

Irrespective of where they differentiate, all flowers are trimerous, zygomorphic and hypogynous. The sepals are thin, glabrous and unequal; two anterior ones are larger being boat-shaped in *C. benghalensis* (Figure 1 *a* and *b*) and obovate in *C. caroliniana* (Figure 1 *c–e*). All flowers have three petals, of which the two posterior petals are large and clawed, with spreading limbs and anterior petal is small linear to lanceolate and pointed at the tip. The petals of chasmogamous flowers are blue, violet and rarely white[3].

The androecium comprises three fertile and three vestigial stamens located at the posterior or lower side (Figure 2 *a* and *b*). The fertile stamens have two types of anthers. The one in the middle has long, inwardly curved, yellow, versatile anther with massive connective (Figure 2 *a*, *b*, *d–f* and *n*). The other two have smaller ovate, dithecous, basifixed anthers raised on bent or curved filaments (Figure 2 *a*, *b*, *d–f* and *n*). These are light grey in *C. benghalensis* and yellow with a brown streak along the line of dehiscence in *C. caroliniana*. The filaments of the stamens are blue that matches with colour of the corolla; the colour is relatively more intense in *C. caroliniana* (Figure 1 *c–e*). Even though the filament length of the two types of stamens does not vary much, greater anther size makes the central/medial stamen slightly longer than the lateral ones and closer to the bent stigma in a floral bud (Figure 2 *a* and *b*; Table 1).

The anther of the central/medial stamen is the largest and that of lateral ones the smallest (Table 1), an invariable trend across the two species. The difference in anther size between stamen types is significant in *C. benghalensis* [$F_{(1,3)} = 98.75$, $P < 0.001$] and *C. caroliniana* [$F_{(1,4)} = 10.76$, $P < 0.05$] for all the flower types [$F_{(3,3)} = 41.87$, $P < 0.001$] in the former. In the latter, this difference is not significant among different flower orders [$F_{(4,4)} = 0.476$, $P > 0.05$].

The length of the filaments of the central and lateral stamens did not vary much (Table 1). However, differences between the sterile and fertile stamens were significant [$F_{(1,4)} = 25.49$, $P < 0.01$ for *C. benghalensis* and $F_{(1,3)} = 105.31$, $P < 0.001$ for *C. caroliniana*] in all flower types and orders [$F_{(4,4)} = 17.62$, $P < 0.01$ and $F_{(3,3)} = 130.85$, $P < 0.001$ respectively].

The vestigial anthers (antherodes) are bright yellow and variously shaped borne on short, slender, light blue to whitish blue filaments. Most of the antherodes are six-lobed. Generally, two middle ones contain pollen grains and the remaining lobes are sterile (Figure 2 *i*, *k* and *l*). However, this is not the rule. Sometimes the antherodes

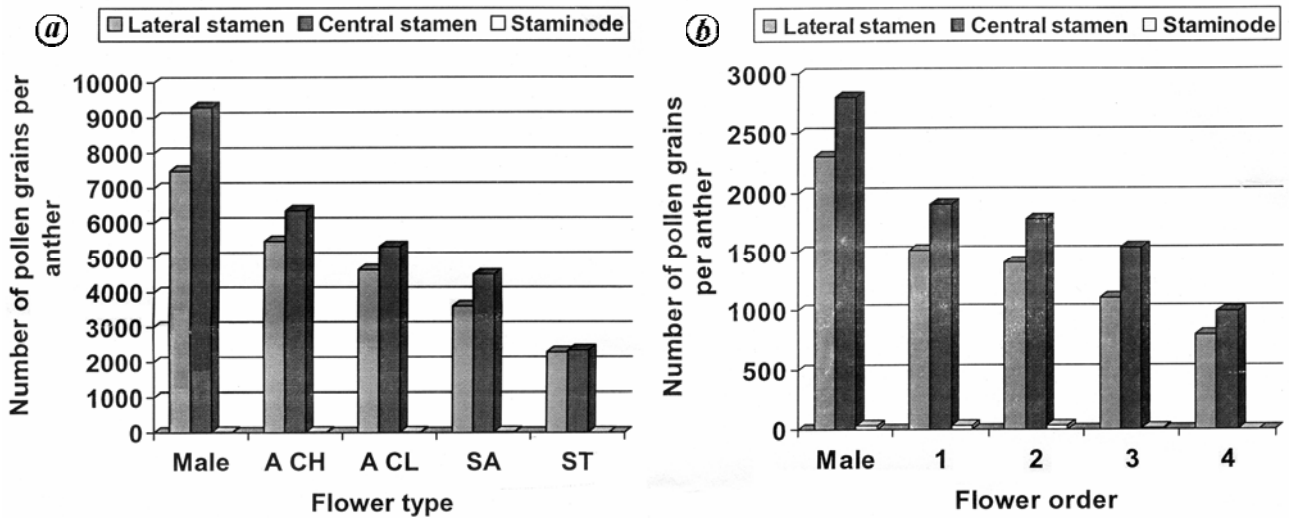


**Figure 3** ***a***, ***b***. Comparative pollen production in fertile (central and lateral) and sterile (staminodes) stamens in (***a***) *C. benghalensis* and (***b***) *C. caroliniana.*

**Table 2.** Data on the size of pollen grains produced by the stamens and staminodes of the studied *Commelina* species

| Flower type/ order | Size (μm) of pollen grains produced | |
|---|---|---|
| | Stamen | Staminode |
| *Commelina benghalensis* | | |
| Male | 32.8 × 21.9* | 34.4 × 23.2 |
| A CH | 30.5 × 21.1 | 32.3 × 22.8 |
| A CL | 28.8 × 19.8 | 30.9 × 22.4 |
| SA | 31.7 × 18.4 | – |
| ST | 25.4 × 20.2 | – |
| *Commelina caroliniana* | | |
| Male | 73.1 × 34.8 | 80.16 × 43.5 |
| 1 | 73.5 × 36.6 | – |
| 2 | 73.5 × 36.1 | – |
| 3 | 67.9 × 34.4 | – |
| 4 | 65.7 × 34.1 | – |

*Average length × average breadth. A CH, Aerial chasmogamous; A CL, Aerial cleistogamous; SA, Sub-aerial; ST, Subterranean flowers.

were totally devoid of pollen and hence sterile (Figure 2 *o*). In some cases only one lobe was fertile (Figure 2 *g*, *h* and *j*). Overall, the staminodes of *C. caroliniana* are much larger than those of *C. benghalensis.* Their size varies between different flowers of a spathe and even within a single flower. Within a staminode the middle lobes were predominantly smallest in size followed by the upper ones; the lowermost were the largest (Figure 2 *g–i*, *l* and *o*). Rarely did the fertile lobes exceed in size (Figure 2 *k*). The alignment of the lobes also varied.

Between different flowers of a spathe, a uniform pattern was found in the two species. Pollen production is highest in the male and went on decreasing from the oldest to the youngest flower on the lower cyme (Figure 3). Between the stamen types, the central stamen is the highest pollen producer irrespective of the flower type and even the species type. Results of two-way ANOVA on log-transformed pollen counts indicate significant variation between flowers of the cyme [$F_{(4,8)} = 7.78$ for *C. benghalensis* and 4.68 for *C. caroliniana*; $P < 0.001$] and among the three types of stamens within a flower [$F_{(2,4)} = 389.8$ and 137.15; $P < 0.001$ respectively]. Pollen production by the staminodes is least ranging from 0 to 68 in *C. benghalensis* and 0 to 54 in *C. caroliniana* (Figure 3 *a* and *b*).

A gradual decrease in size of the pollen grains has been observed from the male flower of the upper cyme to the bisexual flowers of the lower cyme. This is true of both the species. An interesting trend was found; pollen grains of staminodes were larger in size than those of the stamens (Table 2). Increment in size was significant in *C. benghalensis* [$F_{(1,2)} = 144$ for length, $P < 0.001$ and 23.77 for breadth, $P < 0.05$]. The breadth of pollen grains did not vary across flower types [$F_{(2,2)} = 4.84$, $P > 0.05$]; however, the length varied [$F_{(2,2)} = 202.85$, $P < 0.001$]. The same is true for *C. caroliniana* [$t_{(8)} = 3.845$, $P < 0.01$; $t_{(8)} = 13.048$, $P < 0.001$ for length and breadth respectively].

Pollen viability ranged between 81.8% and 87.8% for anther and averaged 78.7% for antherode in *C. benghalensis*. The corresponding values are 80.3–86.8% and 79.2% for *C. caroliniana.* Viability of pollen grains produced by the stamens in both the species was significantly higher than that present in the antherodes [$t_{(8)} = 5.349$, $P < 0.001$ and $t_{(8)} = 3.246$, $P < 0.05$ respectively].

Microscopic examination of hanging slides revealed few or no pollen grains of each of the two species, indicating that plants do not practice wind pollination. As such these results ruled out the role of central versatile anther in pollination, which is normally a contrivance for anemophily.

Flowers, though nectar-less and odourless attract a variety of insects which get lured by the blue petals and filaments, bright yellow stamen and staminodes, purple stigma and moderately wet pollen. About nine hymenopterans visited the flowers of both the species (see refs 9 and 10 for details). Incidentally, the visitors to the two taxa are also common. These are *Nomia eburnigera* and species of *Halictus*, *Nomia*, *Steganomus*, *Bombus*, *Andrena*, *Anthophora* and an unidentified species (for details see refs 9 and 10). Insect visits to flowers extend over a maximum of 6 h a day (Figure 1 *b* and *e*). Species of *Nomia* and *Steganomus* were the most frequent and brisk visitors. Notwithstanding the difference in the size of the individual pollinators, the behaviour was mostly alike across the two taxa. They generally alighted on the male flower and then moved onto the bisexual ones. Once they landed on the flower, the bees first sought the central anther, probed it with their proboscis and legs, and then repeated the same behaviour with the lateral stamens. In this process their body parts get laden with pollen. When the same bee visits another flower, the ventral side of its abdomen and legs brush against the stigma and pollinated it. All the insects collected from the flowers of the two species carried huge quantities of pollen on their different body parts; maximum on the hind legs followed by the forelegs.

However, all these bees were not found gathering pollen from the bright yellow staminodes, indicating the attraction to be deceitful. Flowers emasculated by removing all the three stamens and bagged (treatment I) revealed no fruit set, indicating that the pollen of the staminodes is not capable of self-pollinating the stigma. At a closer examination of such flowers, it was found that the two fertile lobes do not dehisce and pollen present inside is not available for pollination. However, in some of the insect-visited flowers, 'dehisced' lobes were observed. Whether the insects bring about the 'dehiscence' or it occurs on its own and/or its pollen has the potential to cause fertilization, remains to be seen.

Results of treatment II indicate that flowers with only three staminodes did attract insects, but the frequency and efficiency of their visits decreased significantly[10]. This implicates the role of vestigial stamens in attraction by deceit.

Manual pollinations of completely emasculated flowers with pollen collected from both the stamen types of the same (treatment III) or different populations (treatment IV) resulted in good fruit and seed set. However, the fruits harvested were pooled and the quantity sired by pollen of each type could not be determined separately. Completely emasculated and bagged flowers (treatment V) aborted without transforming into fruits. Those which were completely emasculated and tagged (treatment VI) did attract some visitors and resulted into fruit set.

The resulting fruit and seed set following controlled pollinations has been recorded for *C. benghalensis* and *C. caroliniana* (Table 3). Fruit set varies from 70.8% to 100% in different treatments. These figures are high than a mere 66.7–70.0% in treatments I and VI for *C. benghalensis*. In the sister species, the fruit set ranges between 56.7% and 100%; only 40% flowers of treatments I and VI transformed into fruits more than half of which dried and dropped. Seed set showed a similar trend albeit with a greater reduction in treatments I and VI for both the species.

*C. benghalensis* and *C. caroliniana* show striking similarity in the arrangement of stamens and staminodes. Flowers of both the species are heterantherous. The significant difference in size, shape or colour of stamens is an adaptation of flowers for accomplishing the dual functions of attracting and rewarding the biotic pollinators. The anthers of heterantherous flowers that generate pollen for feeding pollinators are called feeding anthers and those which help in promoting pollination are the pollinating anthers[16]. The former being yellow in colour is optically more attractive and the latter are cryptic, being bluish or red. In the present case, the yellow anther of the central stamen and yellow staminodes of *Commelina* flowers appear red to insects; hence they spot them from a distance. No sooner do they alight on the flower, they approach the central anther and fumble with it. Therefore, the lateral stamens are not visited directly. However, their pollen gets deposited onto the body parts of the insects, which harvest food (pollen) from staminodes and possibly the central stamen also. This pollen is then transferred to the stigmas of other flowers.

The amount of pollen produced by the central stamen is also significantly higher than the rest. With the results of hanging slide experiments ruling out wind pollination, all the pollen in this anther is available to the pollinators.

In the absence of nectar, pollen is the only reward that flowers of *C. benghalensis* and *C. caroliniana* offer to the visiting insects. For achieving and ensuring pollinator visitation at minimal cost, flowers of Commelinaceae differentiate conspicuous staminodes, which mimic

**Table 3.** Percentage of fruit and seed set in flowers of *C. benghalensis* and *C. caroliniana* following different pollination treatments

| Species/treatment | Percentage | |
|---|---|---|
| | Fruit set | Seed set |
| *Commelina benghalensis* | | |
| Treatment 1 | 70.0 | 53.0 |
| Treatment 3 | 100.0 | 70.8 |
| Treatment 4 | 100.0 | 86.7 |
| Treatment 6 | 66.7 | 52.8 |
| *Commelina caroliniana* | | |
| Treatment 1 | 60.5 | 45.1 |
| Treatment 3 | 87.5 | 75.2 |
| Treatment 4 | 100.0 | 84.5 |
| Treatment 6 | 56.7 | 38.1 |

pollen-bearing anthers in colour and attract insects by deceit. Once lured inside a flower, pollinators are thought to be rewarded primarily by the pollen provided by the central stamen[1,2,7,8,17]. This has been reported in many species of *Commelina* like *C. communis*, *C. coelestis* and *C. dianthifolia*[1,2,7,8,17], etc. In species like *C. hockii* and *C. forskaolii*, anther of the central stamen is more striking than that of the lateral because of contrasting markings on its broad connective[2]. However, no such markings were found in *C. benghalensis* and *C. caroliniana*.

McCollum *et al.*[18] have treated the central stamen of *C. erecta* as the feeding anther and have mentioned that the pollen contained in it is eaten away by insects and does not, therefore, contribute directly to pollination. While calculating pollen–ovule ratio for the plant, these authors have excluded pollen output of this anther. The pollen produced by the central and the lateral stamens varies in percentage viability, but the seed set following manual pollination by the two pollen types did not vary significantly[18]. Further, pollen produced by the feeding stamens is usually non-viable[19]. In the present case, however, no significant difference was observed in the viability of the pollen that is produced by the lateral and the central stamens. The fundamental role of the central stamen by virtue of its size, shape, colour, placement and orientation may attract and provide a reward for the pollinators. But its importance imparted by the viability of its pollen cannot be underestimated. This is experimentally supported by the high fruit and seed sets in the manual pollinations carried out with pollen from both the stamen types. Whether or not pollen from the central stamen only is capable of causing fertilization and fruit (seed) set has not been assessed. Similar conclusions have been drawn for *C. coelestis* and *C. dianthifolia*[7].

The staminodes are smallest in size, unlike those of *C. coelestis* and *C. dianthifolia*[7], brightly coloured and distinctly lobed. This morphology is quite glaring against the deep-blue corolla, making them discernible from a distance. According to Kevan[20], the combination of blue and yellow lures a wide variety of insects including bees. About nine major hymenopterans and a few more get attracted and frequent the flowers of *C. benghalensis* and *C. caroliniana* at different time intervals and effect pollen transfer.

Pollen produced by anthers of staminodes is neither released from the lobes nor is it ample to be of any use. This is fortified by the results of the pollination treatments. However, as indicated by treatments II and IV, these staminodes do play a small but important role in luring insects for pollination. As their pollen does not participate in gene flow, it has not been included in pollen count estimates[21]. But being a component of floral display, they assist in enhancing pollinator visitation and thereby ensure efficient pollen flow. Furthermore, viability of this pollen is also significantly lower than that found in the stamens. On the contrary, viability of staminode pollen of *C. coelestis* was higher than that of its sister species *C. dianthifolia*, but it failed to set seed after manual pollination[7].

It is quite likely that staminodes of *Commelina* spp. must have evolved from functional stamens. According to Endress[16], heterantherous flowers may be monosymmetric or polysymmetric but, they 'tend to be monosymmetric in some groups of otherwise mainly polysymmetric flowers (e.g. in Commelinaceae, Lecythidiaceae, Gentianaceae, *Solanum*)'. Evans *et al.*[22] also conform to this view. According to them, Commelinaceae flowers are likely to be ancestrally polysymmetric with six fertile stamens, whereas monosymmetry and staminodization are evolutionarily derived[10]. It has been shown that in species of *Commelina* androecial primordial retard early in ontogeny only to develop later and assume their functional role as attractive sterile organs.

Reduced fruit set following emasculation suggests a deleterious effect of the loss of colourful stamens on floral display and resulting pollinator visitation. As *C. caroliniana* flowers are nectar-less, emasculation deprives them of their pollen source. Absence of any reward from the flower is a disincentive to pollinators leading to adverse effect on pollination and fruit set[9]. The same is true of *C. benghalensis*. Although the average fruit set is 70%, seed set was reduced to ca. 50%. Unfortunately, pollinators avoid the emasculated flowers because they forage for pollen. Non-availability of pollen not only reduced the number of visitors, it also drastically cut down the duration of individual visits[10]. Similarly, removal of blue petals artificially from flowers of *C. communis* dramatically reduced approaches by syrphid flies and honey bees[8]. In *Delphinium* also, bumble bees did not visit albino flowers but were frequent to blue coloured flowers[23]. In the present study petals were not removed, although it would be interesting to explore similar possibilities in the other species of *Commelina*, including the two under study. Similarly, to attempt pollination with pollen from all the three anther morphs (staminode, central and lateral stamen) separately is important to assess their siring potential.

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# Biological characterization of lead-resistant bacteria to explore role of bacterial metallothionein in lead resistance

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**Lead-resistant bacterial isolates *Salmonella choleraesuis* strain 4A, *Proteus penneri* strain GM10, *Bacillus subtillis* strain GM02, *Pseudomonas aeruginosa* strain 4EA, *Proteus penneri* strain GM03 and *Providentia rettgeri* strain GM04 were isolated from soil contaminated with car battery waste from Goa, India. All the isolates except *Pseudomonas aeruginosa* strain 4EA showed presence of plasmids. Polymerase chain reaction amplification of 507 bp internal fragment of *smtAB* genes encoding bacterial metallothionein and intracellular bioaccumulation of 19 and 22 mg lead per gram dry weight in *Salmonella choleraesuis* strain 4A and *Proteus penneri* strain GM10 respectively revealed presence of metal-binding metallothionein (SmtA) being responsible for lead resistance encoded by genomic DNA. These lead-resistant and lead-bioaccumulating bacterial isolates can be employed for bioremediation of lead present in contaminated environmental sites.**

**Keywords:** Bacteria, bioaccumulation, lead, metallothionein.

LEAD has a wide range of applications in various industries, viz. petroleum, electronics, battery, paints, ceramics, stained glass, biocide preparation and ammunitions with annual global demand of refined lead exceeding 87 lakh tonnes[1]. Lead, mercury and cadmium are biologically non-essential and toxic heavy metals which affect the terrestrial and aquatic biota along with human beings due to their release from industrial effluents directly into terrestrial and estuarine ecosystems[1,2]. Lead is a persistent environmental pollutant with half life of approximately 5000 years and biomagnifies through the trophic levels. It is important to note that lead causes neurodegenerative diseases, reproductive impairments and renal failure in humans[3,4]. Long-term exposure of humans to lead causes anaemia, cancer, interferes with vitamin D metabolism and causes coma and death if blood level exceeds 70 μg/dl[5–7].

Heavy metal contamination is a major environmental threat worldwide due to their adverse effects (toxicity) on natural biota and humans which is manifested as DNA

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