Reproductive efficiency of *Crotalaria mysorensis* – a vesperal weed of subtropics

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An evolutionary changeover between mating systems comes about frequently and independently in flowering plants. *Crotalaria mysorensis* Roth., an annual weed growing wild in the subtropical regimes of northwestern Himalaya, displays this shift effectively. The species appears as an outbreeder on the basis of its floral architecture. Floral phenological events, however, confirm it to be a strict inbreeder because of its vesperal nature and brief blooming period. This mating strategy provides reproductive assurance to the species, but with low seed germination.

Keywords: *Crotalaria mysorensis*, reproductive efficiency, seed germination, subtropical regimes, vesperal weed.

AN evolutionary shift to selfing from outbreeding is well documented in several flowering plants[1–5]. It is interpreted mainly as an adaptation for reproductive assurance, when conditions are hostile for outcrossing. *Crotalaria mysorensis* Roth., family Fabaceae, displays this shift and constrains efficiently. An annual weed with restricted distribution, the species adopts this strategy by displaying unique phenological events. A population of the species consisting of 50–55 individuals was studied for floral morphology, phenology and reproductive output. Details depicted fruit set by selfing only. This is interesting since most of the species of genus *Crotalaria* studied till date are known to be well adapted to insect pollination. They display anther dimorphism and the interesting mechanism of delayed selfing. The present communication deliberates on the details of this shift.

This study is based on a population of *C. mysorensis* (*n* = 50) sprawling wild at Baba Ghulam Shah Badshah University (BGSBU), Rajouri district, Jammu and Kashmir, India (altitude 912 m amsl, 33°23.654″N lat., 74°20.450″E long.).

Plants were studied for gross vegetative and floral morphology in the field. Data were collected on various aspects like plant height, number of branches/plant, number of leaves/branch, leaf size, number of inflorescences/branch, number of flowers/inflorescence, etc.

To estimate pollen–ovule (P/O) ratio, the pollen count per flower was estimated by squashing the mature undehisced anthers of both types, i.e. long and round (*n* = 20)

in a drop of 1% acetocarmine and counting the number of pollen grains. The number of pollen grains obtained from both types of anthers was multiplied with the number of anthers per flower and then added in order to get the total pollen count per flower. Ovules were counted by gently dissecting them from the locule of the ovary, or by clearing the ovaries at different developmental stages in 5% NaOH for 24 h. After clearing, the ovaries were washed in distilled water, stained in a drop of mixture prepared by mixing 2 ml of 1% acid fuchsin, 2 ml of 1% aqueous light green, 40 ml of lactic acid and 46 ml of distilled water and then mounted in lactophenol and observed under a microscope. The P/O ratio was calculated using the following formula

$$P/O \text{ ratio} = \frac{\text{Pollen count per flower}}{\text{No. of ovules in the same flower}}.$$

Stainability test was performed with 1% acetocarmine, while the viability was assessed by FCR test[6].

To observe the presence of visitors, time of visitation and frequency of visitation on the flower, the plants were regularly monitored in the field throughout the day during the period of full bloom.

To determine the breeding system, pollination experiments were conducted in the field.

Open pollination – Mature and unopened flowers (*n* = 20) were tagged 24 h before anthesis and left for pollination as it happens in nature. These were monitored after a few days to record the fruit set.

Bagging – Mature and unopened flowers and inflorescences (*n* = 20) were covered with butter-paper bags to prevent the visitors and left undisturbed to estimate fruit set after unassisted selfing.

Fruit set and seed set percentage on open pollination and bagging were calculated as follows

Percentage fruit set

$$= \frac{\text{Total no. of fruits formed per inflorescence}}{\text{Total no. of flowers formed per inflorescence}} \times 100.$$

Percentage seed set

$$= \frac{\text{Total no. of seeds per fruit}}{\text{Total no. of ovules in the flower}} \times 100.$$

Seed germination was estimated by sowing the seeds in pots containing garden soil.

Percentage seed germination

$$= \frac{\text{Total no. of seeds germinated}}{\text{Total no. of seeds kept for germination}} \times 100.$$

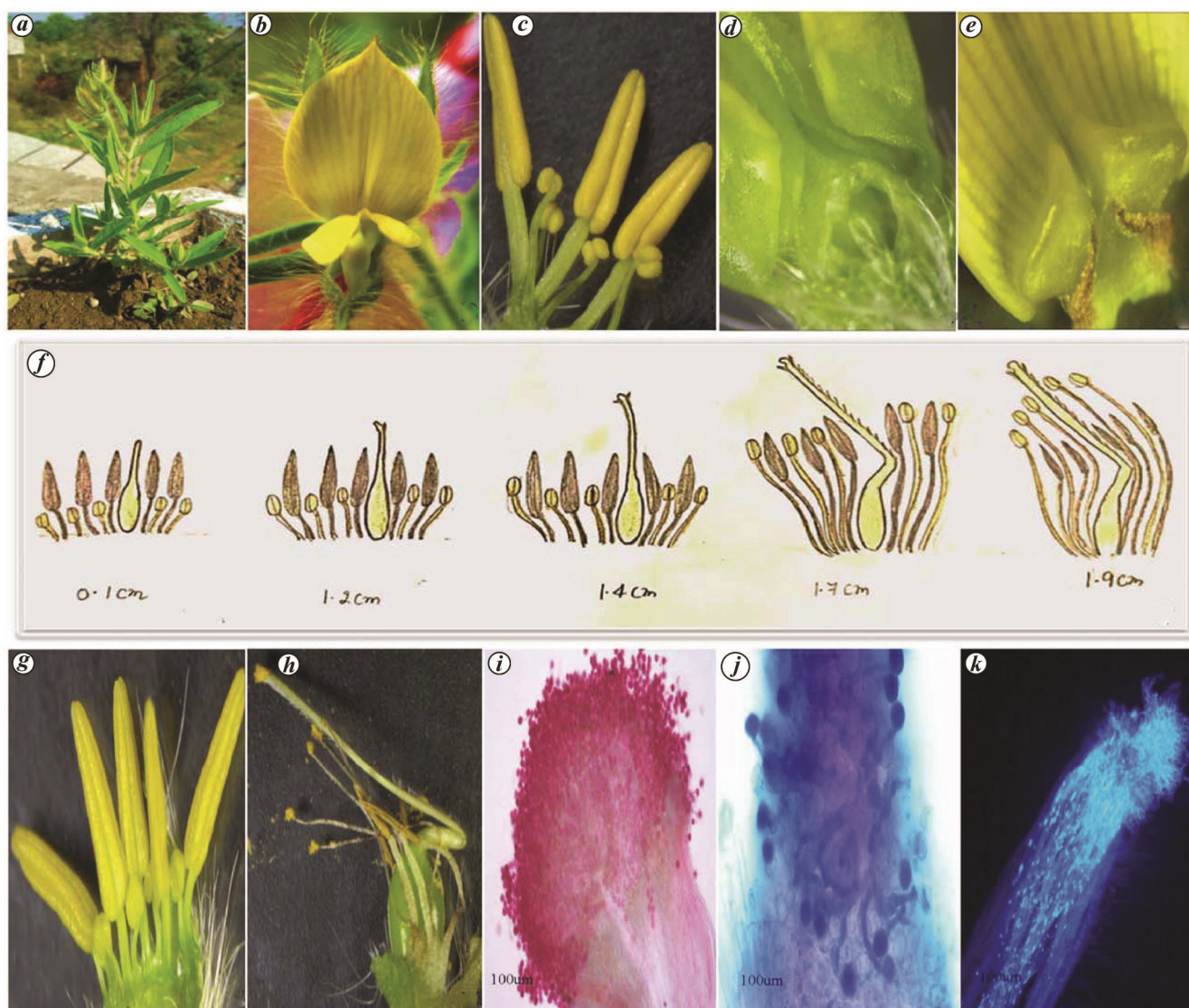*For correspondence. (e-mail: phyllanthus@rediffmail.com)

Figure 1. *Crotalaria mysorensis*. *a*, Plant habit; *b*, An open flower (×1.73); *c*, Arrangement of stamens; *d*, Nectar chamber (×112.1); *e*, Paired callosities on vexillum (×3.90); *f*, Diagrammatic representation of position of anthers vis-à-vis pistil at different development stages in *C. mysorensis*; *g*, *h*, Arrangement of stamens in young and mature flower (×19.4); *i*, Stigma showing pollen load (×19.1); *j*, Pollen germinating on stigma (×16.5); *k*, Fluorescence microphotographs showing path of pollen tubes from stigma through style (×32.1).

C. mysorensis is an annual herb with erect habit (Figure 1 *a*). Plants are 39–51 cm in height (Table 1), with the flowers borne on 5–8 flowered racemes at the end of the branches. Leaves are simple, sub-sessile, stipulate, pubescent, linear–oblong and obtuse, arranged in an alternate manner. They are covered with long spreading hairs on both the sides.

Flowers are papilionaceous, hermaphrodite, zygomorphic, nectariferous and yellow-coloured, characterized by the presence of typical papilionaceous architecture of banner, wings and keel (Figure 1 *b* and Table 2). Calyces are bilabiate-type (3 + 2) forming an upper and lower lip. Like all other species of *Crotalaria*, *C. mysorensis* also shows heteranthery, i.e. presence of dimorphic anthers. Long basifixed and round dorsifixed anthers are present

in 5 + 5 configuration showing distinctive growth of filaments in different phases of flower development (Figure 1 *c*). The pistil is upcurved. Gynoecium consists of unilocular, superior and monocarpellary ovary with glabrous surface, and ovules are attached by marginal placentation. Stigma is brush-type covered with a large number of trichomes. Trichomes are distributed on the style along a single line in *C. mysorensis*. Flowers of this species bear a small round opening at the base of the staminal tube, known as the nectar chamber which encloses nectar (Figure 1 *d*). A pair of ridge-like callosities is present at the bottom of the standard petal opposite the nectar chamber (Figure 1 *e*).

Majority of plants of the population studied enter into the flowering phase during the first week of August each

year, when the temperature in the study area was recorded to be in range 22.03–33.51°C. Plants of this species remain in bloom for a short period of time, i.e. 1.5–2 months only. Flowering is at a peak during the first week of September and ends in the first week of October. After the whole event, plants of this species dry up in November. Anthesis is initiated by the opening of standard petal and it is only the standard petal which opens. This is characterized by the spreading out of the vexillum that deflexes backward exposing the inner wing. Alae slightly separates from the keel that contains essential organs of the flower. Carina opens only if disturbed manually; otherwise it remains closed during the entire flowering period. Flower-opening occurs in the evening hours (4:45–5:30 p.m.) and lasts for 3–4 h only; thereafter the flowers are permanently closed. Detailed observations on floral phenology reveal that in a very young bud, pistil and stamens are nearly equal in height and stigma lies at the level of the anthers (Figure 1 *f*). As mentioned earlier, flowers of *C. mysorensis* possess dimorphic anthers, all of which are fertile. The length of the filament of both types of anthers varies in different stages of flower development (Figure 1 *g* and *h*). In an approximately 0.7 ± 0.0008 cm-long flower, the length of the filament of the stamens having round, dorsifixed anthers is shorter than those having long, basifixed anthers. As the size of the flower increases to 1.5 ± 0.001 cm, filaments of stamens bearing round, dorsifixed anthers becomes three times longer than the filaments of stamens bearing long, basifixed anthers. At the same time, pistil also increases in size and the style develops a curvature below the middle. The curvature of the style causes the stigma to come in close proximity with the round, dorsifixed anthers. There is a difference in the time of dehiscence of the two types of anthers. Long, elongated anthers dehisce 24 h before anthesis, while the round, dorsifixed anthers dehisce approximately 48 h after dehiscence of the long anthers, i.e. 24 h after anthesis.

Flowers are protandrous in the species; however, there is overlapping of the male and female phase within the flower. Stigma attains receptivity approximately 2 h after the dehiscence of long anthers and remains receptive 1:30–2:00 h after the dehiscence of round anthers. Long, basifixed anthers dehisce one day before anthesis, while the small, round, dorsifixed anthers dehisce 24 h after anthesis when the size of the flower averages 1.9 cm. Dehiscence of anthers occurs by the formation of longitudinal slits in both types of anthers. Pollen grains released from both types of anthers are deposited either at the tip of the keel or shed into the keel around the style. A large mass of yellow-coloured pollen is deposited on the stigma from the round anthers. Mature open flowers of *C. mysorensis* have brush-type stigma as it bears a large number of trichomes on either side of its surface. In young buds of size 0.7 cm, stigma has a dome-shape structure and there are no trichomes visible on it. As the size of the bud reaches 1.4 cm, trichomes are seen on either side of the stigmatic surface and at this stage, pollen load averaging 58.4 ± 0.43 is seen deposited on stigma (Figure 1 *i*). The stigma shows maximum receptivity in a flower of size 1.9 cm. At this time, it shows high pollen load from the round anthers averaging 931.25 ± 11.71, 48.42% of which is seen germinating (Figure 1 *j*). Fluorescence microscopy of pistil done at this stage shows that several pollen tubes traverse the stylar tissue and reach the ovule without any hinderance throughout their pathway (Figure 1 *k*). Pollen tubes enter the ovule at the micropylar end. No insects were seen visiting the flowers of *C. mysorensis* in the 1.5–2 months of observation.

Pollen output per long anther was much higher compared to the round anther (Figure 2). Total pollen count/long and round anther was 19201.75 and 6770.45 respectively, whereas ovule count ranged from 31 to 56. Thus, pollen count as well as ovule count was high in *C. mysorensis*. P/O ratio was 2797.04 : 1. Based on the P/O ratio of Cruden[7], *C. mysorensis* tends to fluctuate between 'facultative xenogamy and obligate xenogamy'.

Reproductive output of *C. mysorensis* in terms of fruit set on bagging as well as open pollination was 100% (Table 3).

Fruit is a pod and it is inflated, oblong and glabrous. Seeds are reniform, compressed, beaked at one end and

Table 1. Vegetative morphometry of *Crotalaria mysorensis*

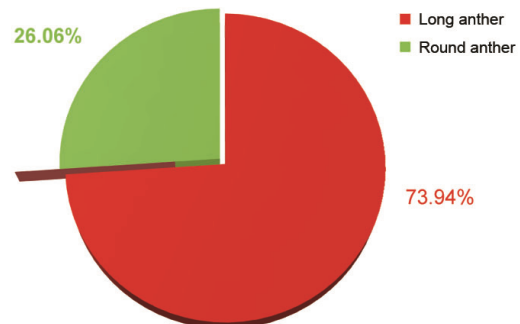| Characters (*n* = 20)	Average	Range
Plant height (cm)	45.1 ± 0.97*	39–51**
Length of internode (cm)	1.7 ± 0.04	1.4–2
No. of branches/plant	3.3 ± 0.25	2–5
No. of leaves/plant	51.1 ± 1.60	42–64
No. of leaves/branch	9.7 ± 0.27	9–12
Length of leaf (cm)	6.1 ± 0.05	5.8–6.6
Width of leaf (cm)	1.3 ± 0.03	1.1–1.6
Length of stipule (cm)	1.1 ± 0.01	1.1–1.2
Width of stipule (cm)	0.20	–
No. of inflorescences/plant	2.7 ± 0.15	2–4
No. of flowers/inflorescence	3.4 ± 0.18	2–5

*Mean ± standard error. **Range.

Figure 2. Percentage of pollen grains produced by long and round anthers in *C. mysorensis*.

Table 2. Floral morphometry of *C. mysorensis*

Characters (*n* = 20)	Average	Range
Length of inflorescence (cm)	9.35 ± 0.13*	9–11**
Length of pedicel (cm)	0.94 ± 0.01	0.9–1.1
Length × width of flower (cm)	2.3 ± 0.002 × 0.705 ± 0.005	(2.3–2.36) × (0.7–0.8)
Androecium		
Length of staminal tube (cm)	0.38 ± 0.01	0.3–0.5
Length of stamen of long anther (cm)	1.29 ± 0.005	1.2–1.3
Length of stamen of round anther (cm)	1.99 ± 0.006	1.9–2
Length × width of long anther (um)	3000.05 ± 5.17 × 439.9 ± 0.61	(3000–3001) × (19–37)
Length × width of round anther (um)	322.87 ± 5.17 × 251.58 ± 10.82	282.2–348.6
Gynoecium		
Length × width of ovary (cm)	0.6 ± 0.002 × 0.3 ± 0.04	(0.6–0.65) × (0.3–0.6)
Length of stigma + style (cm)	1.4 ± 0.003	1.4–1.46

*Mean ± standard error. **Range.

Table 3. Data on fruit and seed set in *C. mysorensis*

	Characters	Percentage
Open pollination	Percentage fruit set	100
	Percentage seed set	99.79
On bagging	Percentage fruit set	100
	Percentage seed set	98.84

shiny black in colour. Single fruit contains 30–50 seeds. Pattern of fruit set clearly indicates the mode of breeding system in this species. Percentage seed set is also high in the species. However, germination of seeds is low (42.5%).

Like most species of *Crotalaria*, in *C. mysorensis* also the flowers are papilionaceous, show heteranthery, being protandrous with pollen deposition by anthers onto the style and keel before the stigma becomes receptive. Such a temporary separation of male and female, i.e. dichogamy is an indication of the outbreeding mechanism[8,9]. The species also shows several other adaptations for insect pollination like coloured flowers, floral architecture and a good amount of pollen and nectar as reward. Sequence of anther dehiscence as well as stigma and style curvature in the species are typical of delayed selfing adaptation of genus *Crotalaria*. In this mechanism, initially pollen is isolated from stigma to prevent autogamy and to encourage outcrossing by biotic pollinators. Later, if the flower is not visited by any pollinator and cross-pollination fails to occur, selfing occurs. Since selfing is the second option, the term 'delayed selfing' is used. Staggered dehiscence of two types of anthers and elongation of filaments to reach the stigmatic surface are two important components of this mechanism.

Pollen released by long anthers is pushed into the tip of the keel by the elongation of round, dorsifixed anthers and is available for pollinating its own stigma. Dehiscence of round anthers at this juncture close to stigmatic surface attenuates the selfing process. Small, round anthers therefore play an important role. They cooperate with the stylar brush for pollen presentation and also help deliver the remaining pollen to the stigmatic surface almost near the end of the receptivity period[10]. It can be interpreted as a counterstroke to the degree of unreliability of biotic agents[11–13].

Interestingly, pollen size differs for long and round anthers in the species. Functional significance of this could not be ascertained in the present study. Dimorphism in pollen has also been reported in several *Crotalaria* species, including *C. medicaginea*, *C. quartiniana*, *C. goreensis*, *C. incana* subsp. *inacana* and *C. incana* subsp. *purpurescence*[14].

Unlike most of species of *Crotalaria* which are diurnal, *C. mysorensis* is vesperal in nature with a short span of blooming, i.e. 1.5–2 months in terms of bloom and 3–4 h in terms of flowering. Due to these factors, insects elude this plant. As a result, the dehisced anthers and stigma remain enclosed in the carina. As the stigma attains receptivity, it remains bereft of pollen; however this temporal separation is not complete. As the second set of anthers dehisce, pollen grains become available to self-stigma[15]. Presence of two types of anthers, their staggered dehiscence and elongation of filaments making the anthers to contact the stigma are mechanisms that assure successful fertilization and seed set. On the second day, the round anthers contact the receptive stigma and dehisce, resulting in self-pollination. The species seems to have adopted this mode. Thus fertilization is delayed, but is assured as it sets the highest percentage of fruits even in the absence of insects and shows no decline in fruit set when bagged. One reason to shift itself from outcrossing towards selfing can be the annual life cycle of the species, which might also give away this species to the risk of temporary pollinators[16].

C. mysorensis is purely an inbreeder. The dehisced anthers and stigma remain enclosed in the carina as insects dodge these plants. In the beginning of receptivity period, stigma remains devoid of pollen; however on the second day of receptivity as the filaments of the round anthers grow substantially and reach the proximity of the stigma, self-pollination occurs. The species sets highest

percentage of fruits even in the absence of insects and shows no decline in fruit set in bagged inflorescence. Outcrossing is interestingly evaded by *C. mysorensis* as flowers are vesperal in nature, open between 4:45 and 5:30 p.m. for just 3–4 h and there are no insect visits.

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Macro-charcoal in carbonaceous strata of the Lower Cretaceous of northwest India: remains from the Than Formation, Saurashtra Basin, Gujarat

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The record of Cretaceous palaeo-wildfires is rather scarce for peninsular India. We aim to report a detailed macro-charcoal analysis as evidence for palaeo wildfires of Early Cretaceous deposits from India. The macro-charcoal was studied using SEM and classified into three morphotypes based on anatomical characteristics. All morphotypes are probably associated with gymnosperms. These findings constitute a record of macro-charcoal and consequently of palaeo-wildfires for the Lower Cretaceous strata of the Saurashtra Basin, Northwest India.

WILDFIRES are common events in modern and past ecosystems, being a significant evolutionary driver of biodiversity and ecosystem dynamics since the Silurian[1–3]. After fire, plants that did not undergo complete combustion may be preserved as charcoal which can be incorporated in the sediments, and thus can provide direct evidence for the occurrence of palaeo-wildfires in recent and deep time environments[4]. The study of such records enables conclusions concerning the composition of the vegetation affected by fire and some of the palaeoenvironmental conditions of the surrounding areas[5]. Considering fossil macro charcoal, only a few records have been published for Indian strata and they mostly originate from the Permian deposits[6–10]. Although the Cretaceous is globally considered a high-fire interval[11,12], only a single macro-charcoal[13] and two micro-charcoal[14] occurrences have been published so far for that interval from India. Taking into account the current lack of data about palaeo-wildfire occurrences for the Cretaceous of India and the

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