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Pollination mechanism and pollinators of the endemic plant *Rhynchosia beddomei* Baker (Fabaceae)

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Abstract
Rhynchosia beddomei flowers during December-March with peak flowering during January. The flowers are hermaphroditic, nectariferous, self-compatible and have explosive pollination mechanism adapted for pollination by bees, especially carpenter bees. The flowers not visited by bees fall off while those visited and pollinated by them set fruit. Therefore, *R. beddomei* is obligately dependent on pollinating bees for pollination which occurs due to tripping of the explosive mechanism.

Keywords: *Rhynchosia beddomei*, explosive pollination mechanism, melittophily

1. Introduction
The genus *Rhynchosia c*onsists of approximately 200 species and occurs in both the eastern and western hemisphere in warm temperate and tropical regions (Grear 1978) [1]. In the Eastern Ghats, twelve species of this genus have been reported to be occurring almost in one region, Seshachalam hills of southern Eastern Ghats of Andhra Pradesh. They include *R. beddomei, R. rufescens, R. suaveolens, R. cana, R. albiflora, R. capitata, R. courtollensis, R. densiflora, R. heynei, R. minima, R. rothii, R. rufescens, R. suaveolens* and *R. viscosa*. These species are either climbers or shrubs (Madhava Chetty *et al.* 2008) [2]. Of these, *R. beddomei* is a rare and endemic medicinal species and restricted to a few areas such as Talakona, Japalitheertham, Gogarbham in Seshachalam hills of Chittoor District, Andhra Pradesh (Padmavathi *et al.* 2012) [3].
Madhav Chetty *et al.* (2008) [2] reported that *R. beddomei* is useful for certain purposes such as abortifacient, anti-bacterial, anti-fungal, diabetes and hepato-protectivity. Rama Rao and Henry (1996) [4] noted that the leaves of *R. beddomei* are used for wounds, cuts, boils and rheumatic pains by Adivasi tribes inhabiting the forests of Eastern Ghats of Andhra Pradesh, India. Gunasekar (1984) [5] noted that this plant is contains flavonoid compounds, such as flavones, flavonols and flavanones. Bakshu and Venkata Raju (2001) [6] mentioned that the leaves of this plant possess significant antimicrobial activity. These various medicinal uses by locals might have led to the endemic status of this shrub. Since the plant is now an endemic in the southern Eastern Ghats, its pollination mechanism and pollinators have been observed.

2. Materials and Methods
The study region is the southernmost region of Andhra Pradesh and is an integral part of Southern Eastern Ghats of Peninsular India. The area is located at 13°40'N latitude and 79°19'E longitude, and at an elevation of 2,443 ft. The exact study area is the forest cover of Tirumala Hills a constituent of Seshachalam Hill Range in Chittoor District, Andhra Pradesh. The entire region represents the deciduous forest ecosystem. The site is characterized by a combination of rocky, undulating and steep terrain with some litter content formed from grass and other herbaceous plants. This forest is known as hot-spot for some rare, endangered, vulnerable, threatened and endemic plants (Madhava Chetty *et al.* 2008) [2]. *Rhynchosia beddomei* was examined in the field for its floral biology, pollination mechanism and pollinators. Flowering season was defined based on regular field trips made. Ten plants were tagged and followed for quantifying the flower production rate daily. The flower count was computed and provided week-wise during flowering season. Twenty inflorescences were tagged and followed to record the length of flowering and the number of flowers produced. Observations regarding the organization of inflorescences, the spatial positioning of flowers, and their position (terminal, axillary, etc.) on the plants were made since these features are regarded as important for foraging and effecting pollination by flower-visitors. The flower life was recorded by marking twenty five just anthesed flowers and following them until fall off. Anthesis was initially recorded by observing twenty five marked mature buds in the field. Later, the observations were repeated five times on different days in order to provide accurate anthesis schedule. Similarly, the mature buds were followed for recording the time of anther dehiscence. The presentation pattern of pollen was also investigated by recording how anthers dehisced and confirmed by observing the anthers under a 10x hand lens. The details of flower morphology such as flower sex, shape, size, colour, odour, sepals, petals, stamens and ovary were described based on twenty five flowers randomly collected from five plants.
After making preliminary observations on flower visitors, the categories of insects were identified. The flower foragers included only bees and they were recorded. Fully blooming plants were selected to record the foraging visits of bees. The role of pollinators in tripping the pollination mechanism has been examined in detail.

3. Results
It is an erect, perennial shrub, 1.5 m tall with tomentose branchlets that grows in rocky areas with red soils. The plant re-grows from below ground perennial root stock and from

the seed during wet season from July to November during which growth and leaf flushing occurs. The leaves are trifoliate with reticulate venation. The leaflets are ovate-lanceolate, slightly silvery, silky and coriacious. The flowering occurs during December-March with peak flowering in January. The plants wither and disappear in April. The flowers are borne in pedunculate axillary and terminal racemes; individual racemes are 5-8 flowered which open over a period of 3-5 days.

The flowers are small, yellow, odorless, papilionaceous, zygomorphic and bisexual. The calyx is green with purplish tinge and consists of 5 free oblong, obtuse sepals; the upper two sepals are longer than the lower 3 sepals. The corolla is bright yellow, pubescent, specialized and consists of upper standard petal, two wing petals and two keel petals. The standard petal is large, obovate with reddish-brown lines at the bottom of the mid-region which serves as nectar guide; the petal base is clawed and consists of two inflexed fingernail auricles. The standard petal envelops the rest of the petals in bud but reflexes when the flower blooms. The two adjacent petals, called wing petals surround the two bottom petals, called keel petals. The keel petals form a proximal cylindrical part and a distal part consisting of a pressed angular pouch, with an acute porate tip in which the stamens and stigma are housed. The keel and the wing petals are attached by means of two notched folds. The wing petals serve as alighting platform for insects visiting the flowers. The stamens are ten, diadelphous; nine filaments are fused by the basal part into a sheath open along the upper side while the tenth filament is free and lies on the others. The distal parts of the filaments are free and contain 1 mm long uniform dithecous anthers. The ovary is sessile, green, villous, and lies in the sheath of the filaments along the cylindrical part of the keel. It is monocarpellary and monolocular with a single ovule arranged on marginal placentation. It has a long glabrous style with a capitate wet shiny stigma. The stigma is situated slightly above the anthers. The distal portion of free filaments and style and stigma are incurved and clamped into the keel petals.

Mature buds open during 1200-1500 h. Unfolding of the standard petal and wing petals indicates flowering opening. The keel petals do not unfold and remain in their original position as in mature bud stage. All the ten anthers in a flower dehisce at the same time by longitudinal slits in mature bud stage. The reproductive column is held under pressure within the keel part in open flowers and it is exposed when the pollinator presses against the wing and the keel petals. When insects land on the wing petals, the latter causes the keel petals to release the reproductive column explosively. Consequently, the reproductive column snaps forward against the standard petal causing most of the pollen to be instantly released and the pollen thus released comes into contact with the ventral side of the insect body. Since the incurved stigma is situated above the height of the anthers, it strikes the insect body first due to which cross-pollination occurs if the insect visited the other flowers previously and carried pollen on its ventral side and also then the pollen ejected from the anthers powders the ventral side of the insect instantly. If it is the first visit for the insect to the flower, then it effects self-pollination upon explosive release of reproductive column from the keel boat. With the departure of the insect from the flower, the reproductive column does not return back to its former position but the keel moves forward partly covering the stamens and stigma. The downward movement of keel petals occurs in each subsequent foraging visits by appropriate insects. Tripping of keel boat can also occur due to heavy rain or high temperature that weaken turgidity of the restraining keel tissues. But, the tripping due to these two factors is ruled out since the plant flowers during winter season when heavy rains do not normally occur and the temperature usually stands low. If the flower is untouched or tripping to keel did not occur, the reproductive column is never exposed and remain enclosed in the keel boat. Such flowers fall off subsequently upon withering without fruit set.

Insect activity was not found at the inflorescences during forenoon period. Their activity was recorded only from noon time onwards due to the availability of fresh flowers. Insects that visited the flowers belonged to only one order, Hymenoptera, one family, Apidae and three sub-families, Apinae, Nomiinae and Xylocopinae, all belonging to bee category. Four bee species belonged to Apinae, one species to Nomiinae and two species to Xylocopinae. The bees included *Apis dorsata, A. cerana, A. florea, Ceratina* sp., *Nomia* sp., *Xylocopa pubescens* and *Xylocopa* sp. The last two bee species were exclusive nectar foragers while all other bee species were both pollen and nectar foragers. The flowers were visited several times by bees but new visits lasted shorter than the first one. On certain occasions the bees abandoned their intention of browsing on previously visited flowers upon landing. With respect to their their behavior, the bees landed on the wing petals and the keel, with their head near the standard. They then exerted a certain pressure with legs on the wing petals until these and the keel bent downwards, and then proceeded to collect nectar during which the bee's abdomen appeared pollen smothered (sternotribic pollen deposition). The pollen collecting bees took "U" turn after nectar collection and proceeded to the stamens to collect pollen.

Table 1: List of bee foragers on *Rhynchosia beddomei*

Order	Family	Sub-family	Genus	Species	Common Name	Forage collected
Hymenoptera	Apidae	Apinae	*Apis*	*dorsata* F.	Rock honey bee	Nectar + Pollen
			Apis	*cerana* F.	Asiatic honey bee	Nectar + Pollen
			Apis	*florea* F.	Dwarf honey bee	Nectar + Pollen
			Ceratina	sp.	Small carpenter bee	Nectar + Pollen
		Nomiinae	*Nomia*	sp.	Alkali bee	Nectar + Pollen
		Xylocopinae	*Xylocopa*	*pubescens* Spinola	Large carpenter bee	Nectar
			Xylocopa	sp.	Larger carpenter bee	Nectar

4. Discussion

Within the sub-family Papilionoideae, primary and secondary pollen presentations have been reported. In plants with primary pollen presentation, pollen is delivered directly from the anthers to the vector's body. In plants with secondary pollen presentation, pollen grains are delivered first on a floral part such as the keel petals in Papilionoideae and then on the body of the vector implying an accurate delivery of pollen on the vector's body (Howell *et al.* 1993) [7]. These two pollen presentation patterns are associated with the four types of basic pollination mechanisms - valvular, pump, explosive and brush, all of them are associated with a particular floral architecture and kinetics. In the valvular type, pollen presentation is primary, whereas in the other three mechanisms, it is secondary (Yeo 1993) [8]. In the explosive mechanism, commonly only one pollination event occurs and it has evolved independently in several tribes (Small 1988) [9], while in the other three mechanisms, repeated visitation is possible (Westerkamp 1997) [10]. In the present study, *R. beddomei* flowers have explosive pollination mechanism and deliver pollen directly from the anthers to the bee's body when keel petals are tripped by the foraging bee; this type pollen delivery is the representative of primary pollen presentation associated with explosive pollination mechanism. In the flowers, the staminal column is held under pressure within the keel, and when the tension is released by the forager, the same column snaps forward against the standard petal causing all the pollen to be instantly released. The reproductive column remains exposed and does not return back to its original state but the keel petals return back partially covering the stamens and stigma. The efficiency of explosive pollination mechanism depends on the ambient weather conditions, especially temperature and relative humidity.

In *R. beddomei*, the keel tripping process is not self-activated to effect pollination. The flowers depend on bees for tripping of the keel petals to enable the working of explosive pollination mechanism. The flowers that were not tripped by external agents subsequently fall off. This situation explains that the plant is obligately dependent on bees for pollination. Of the bees, carpenter bees and the rock honey bee being large in size are more efficient in tripping the flowers than other bees. Carpenter bees are also more efficient in lifting the flag petal to access the nectar situated at the flower base. Since these bees collect only nectar and more efficient tripping the flowers to effect pollination, they are classified as principal pollinators. All other bees although trip the flowers and effect pollination are treated as next-rank pollinators because they reduce the availability of pollen by pollen collection. The scarcity or non-availability of reliable floral resources during winter season in the study area further enforces fidelity to *R. beddomei* by bees, in particular pollen collecting ones due to which the pollen availability for pollination gets very much reduced. Mishra and Rajesh Kumar (1997) [11] reported that the pollen has great importance for a bee colony as pollen provides proteins, which are essential for worker honey bees to secrete glandular food (royal jelly) for rearing brood. Availability of enough pollen directly helps in more brood rearing, which ultimately leads to gradual colony build up. *R. beddomei* is a promising source of pollen for honey bees and other bees during winter season. Therefore, *R. beddomei* despite having specialized pollination mechanism with primary pollen presentation adapted to bee pollinators is unable to populate itself due to several limitations during growth season.

5. References

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