

Trichodesma zeylanicum: an unusual pollination system with chasmogamous flowers and obligate autogamy

Flowering plants show great variation in their mode of pollination. A small proportion of plants produces cleistogamous flowers. As they do not open, their pollination is exclusively autogamous (with pollen from the same flower); there is no possibility of geitonogamous (pollen from another flower of the same plant) or xenogamous (pollen from another plant) pollinations^{1,2}. A majority of flowering plants, however, produce chasmogamous (open) flowers and permit both self- and cross-pollination. Dioecious and strictly self-incompatible species are obligately xenogamous (pollen has to come from other plants). Most of the remaining chasmogamous species show a mixed mating system in which both self- and outcross-pollen contributes to pollination success, although the extent of each varies greatly depending on the floral structure and the pollination environment.

Autogamous self-pollination is one of the frequent methods of reproductive assurance evolved to cope with uncertain pollinator environments³⁻⁷. Although autogamy is present in a range of species spread across flowering plants, it appears to be a major adaptation, particularly in annual weed species⁸. As annual weeds do not have vegetative propagation and can produce seeds only once in their life, autogamy provides reproductive assurance, even in the absence of pollinators, which is crucial for their survival and spread. However, autogamy is not critical in perennial weed species as they are able to produce seeds repeatedly during their life; even if they miss seed production in some years, their survival is not at risk. Here I report a novel pollination system in a perennial weed, *Trichodesma zeylanicum* (Boraginaceae), which produces typical chasmogamous flowers, but pollination is exclusively autogamous.

Studies were conducted during two flowering seasons (2013 and 2014) on plants growing on fallow lands at Odekar farm (13°32'35"N, 77°2'53"E, elevation 825 m msl) located about 20 km from Tumkur, Karnataka, South India. Floral phenology was studied by tagging the flower buds ($N=30$) and monitoring them from anthesis (flower opening) until senescence. The extent of autogamous self-pollination was studied in

bagged flowers. Another set of flowers was tagged and allowed to open-pollinate. A third set of flowers was emasculated (by pulling out anther cone along with the corolla from just opening flowers before anther dehiscence) and bagged to check the presence of apomixis. One set of bagged and open-pollinated flowers was excised after the corolla and anther cone abscised and their stigmas observed under a stereomicroscope for the presence of pollen grains. Some of the pollinated pistils ($N=6$) were preserved in 70% alcohol and used to study pollen germination and pollen tube growth using aniline blue fluorescence method⁹. The remaining flowers (both bagged and tagged) were left on plants to record fruit set. Floral visitors were monitored at 30 min time slots each hour in the morning (06.00–10.00 h) and evening (14.00–18.00 h) for four days during the two flowering seasons, totalling 16 h of observation. The differences in pollination efficacy in terms of the proportion of flowers pollinated and those that set fruits (%) between the bagged and open-pollinated flowers were analysed by pair-wise *t*-test using SPSS 16 statistical package (SPSS Inc., Chicago, USA).

T. zeylanicum (Burm. F) R. Br. (Boraginaceae) is an erect undershrub covered with dense, hispid hairs on the surface. Flowering is initiated in the first week of December and continues until the end of

April. Flowers are borne in clusters at the tips of the branches. Flowers are pendulous (Figure 1a); they measure about 1.2 cm in length and 1.4 cm in diameter. Five green sepals fuse at the base to form a cup and the lobes are free at the tip. The five petals are fused to form a basal tube of 4–5 mm and expanded terminal part to form bell-shaped pale blue corolla (Figure 1b). There are five small grooves present at the base of the expanded petals leading to the corolla tube. Stamens are five without any filaments and epipetalous. Each anther is compactly appressed laterally to the neighbouring anthers to form an anther chamber at the centre in which the style and stigma are located. Anthers are introrse and the tip of each anther elongates in the form of a narrow filament. The terminal parts of the filaments of all anthers are twisted spirally to form a pointed cone of 5–6 mm, projecting from the centre of the corolla (Figure 1c). Anther cone never opens and thus the pollen-bearing parts of anthers and the stigma are not exposed during the active life of the flower. Each anther produces dense, compactly arranged white hairs on the outer surface on both the sides along its length; the hairs of the neighbouring anthers intertwine to form a tight mesh. Because of these hairs, considerable force is required to separate individual anthers. The pistil is 7–8 mm long, terminating in a capitate stigma located at the tip of the stamen cone

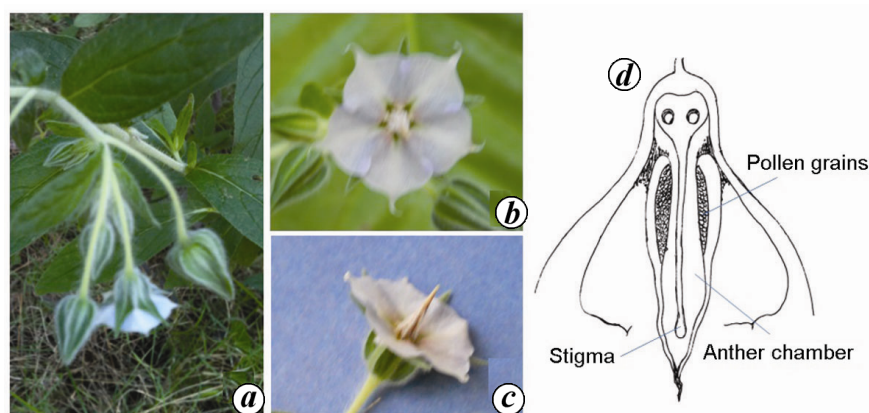


Figure 1. Floral features of *Trichodesma zeylanicum*. *a*, Flowering branch to show pendulous flowers. *b*, Top view of the flower. *c*, Side view of the flower to show anther cone. *d*, Diagrammatic representation of longitudinal section of the flower to show details of anther chamber, location of pollen grains in dehiscent anthers and pistil.

Table 1. Pollination efficiency and fruit set in bagged and open-pollinated flowers

Treatment	Open-pollinated flowers		Bagged flowers		<i>t</i> value
	<i>N</i>	No. of pollinated/set fruit (%)	<i>N</i>	No. of pollinated/set fruit (%)	
Pollination efficiency	23	21 (91.3)	25	22 (88.0)	$t_2 = 1.337, P = 0.3142$; NS
Fruit set	42	39 (92.85)	50	46 (92.0)	$t_4 = 0.169, P = 0.873$; NS
Emasculated and bagged flowers			25	0	

about 1–1.5 mm above the tips of the anthers. Ovary is four-loculed with one ovule in each locule. There is no visible nectar in the flowers. Fruits are four-seeded nutlets. The sepals are persistent; they dry up and open out in the form of wings in mature fruits and may help in their dispersal.

Opening of the flowers is not synchronous; they start opening from the early morning (06.00 h) and continue until 14.00 h. The anthers dehisce longitudinally about 3–4 h after anthesis exposing white, powdery pollen mass on the inner surface of the anther chamber (Figure 1 d). The corolla abscises at the base, 24–36 h after anthesis, and drops off along with the anther cone leaving the style and stigma intact. The architecture of the flower does not permit floral visitors to have access to the pollen or the stigma that is enclosed within the anther chamber and thus are not able to bring about pollination. Also, floral visitors do not get any reward. No floral visitors were observed during 16 h of observation spread over four days. The only possible means of pollination is through autogamy.

To check the extent of autogamy, bagged flowers and those allowed for open pollination were studied for pollination efficacy and fruit set. In both the sets, about 90% of the flowers got pollinated (Table 1). There was no significant difference in the extent of pollination in the two sets of flowers. As the flowers are pendulous and get disturbed by prevailing wind, some pollen grains are likely to fall on the stigma due to gravity. Further, when the corolla abscises at the base and falls off along with the anther cone, the stigma invariably comes in contact with the pollen-laden inner surface of the anthers and is likely to get additional pollen load. The extent of fruit set in bagged and open-pollinated flowers was similar and there was no significant difference between the two (Table 1). None of the emasculated flowers set fruits, indicating absence of apomixis in the species.

The results clearly show that there is no possibility of animal-mediated pollination or wind pollination in the population of *T. zeylanicum* used in the present study. Pollination biology of three other *Trichodesma* species, *T. africana* and *T. boissieri*¹⁰ and *T. indicum*¹¹, has been studied. In all these species, the flowers offer nectar and the tip of the anther cone opens through which the stigma emerges, thus making it accessible to the pollinators. The flowers in all these species are also pendulous and show typical buzz pollination in which the pollinators, largely Hymenoptera, vibrate the flowers during their visit forcing the pollen from the anther chamber to land on their body; when the pollen-loaded insect visits the flower with protruded stigma, pollination is achieved.

The other species which shows some similarity to the flowers of *T. zeylanicum* is *Wrightia tinctoria* (Apocynaceae)¹². The pollen and the stigma, located just below the anthers of *W. tinctoria*, also remain concealed inside the cone formed by the stamens. However, the filaments are not fused with each other leaving a wide gap at the base that gradually narrows towards the tip. The flowers provide nectar as reward. Several insects, particularly of Apidae, visit the flowers and insert their proboscis through the openings of anther filaments to harvest the nectar. While leaving the flowers, the proboscis of some insects gets stuck in the narrow gap between the filaments toward the tip of the cone. The insects struggle to get themselves free and during this process disturb/vibrate the flower and bring about autogamous pollination¹². Thus, *W. tinctoria* is also obligately autogamous, but it is facilitated by floral visitors.

T. zeylanicum provides a unique pollination system; although it produces typical chasmogamous flowers, the pollination is exclusively autogamous and is not dependent on insect visits. Pendulous flowers and abscission of the corolla along with anther cone facilitate autogamy. To

my knowledge, there is no other example of autogamous pollination comparable to *T. zeylanicum*. Pollination systems are evolutionarily labile and may shift rapidly in response to local conditions³. Other populations of this species need to be studied to determine whether this feature is present across the species or limited to some populations.

Selfing lineages are considered to have short-term advantageous because of their ability to reproduce, colonize and expand under conditions of low density of conspecific plants and uncertain pollinator environment^{2,3}. However, such lineages are likely to be short-lived owing to higher rates of extinction because of their reduced ability to adapt to environmental changes as a result of increased homozygosity¹³. Due to these reasons, Stebbins¹³ considered obligately selfing species as an evolutionary 'dead end'. In general, most of the selfing species show mixed mating system; although they are predominantly selfers, they do permit cross-pollination when pollinators are available. Even the species that produce cleistogamous flowers bear some chasmogamous flowers permitting cross-pollination. In fact, it has been suggested that very few plant species, if any, show complete selfing (see Wright *et al.*⁷). *T. zeylanicum* is a rare example of exclusive selfing and provides an interesting system to study long-term effects of selfing.

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Assessment of endolichenic fungal diversity in some forests of Kumaun Himalaya

The statement by Hammer¹ that ‘Biodiversity studies depend upon biogeography and biogeography depends upon biodiversity’, emphasizes that without insights into biogeographical patterns we cannot fully understand the evolution of species and without some knowledge of what grows where, our attempts at something as simple as identification may prove fruitless. Henceforth, if we accept Hawksworth’s hypothesis² that there are 1.5 million species of fungi known from the world of which only 100,000 are described, then a question arises ‘Where are all the undescribed fungi?’ Hawksworth & Rossman³ identified three categories where we can find these undescribed species: (1) fungi in tropical forests, (2) fungi in unexplored habitats, and (3) lost or hidden species. The second category (fungi in unexplored habitats) includes hypogeous fungi in Australia, fungi in the guts of other beetles and insects, lichenicolous fungi and endophytic fungi.

Endophytes are organisms which live inside other organisms without producing any apparent disease symptoms. They are a polyphyletic group of highly diverse, primarily ascomycetous fungi defined functionally by their occurrence within asymptomatic tissues of plants^{4,5}, mosses and ferns^{6,7}, marine algae^{8,9}, and seed plants from the Arctic to the tropics, and from agricultural fields to the most biotically diverse tropical forests. Their population depends on host species, location and environmental conditions in which the host is growing¹⁰. Commonly, a single plant can be a host of numerous

endophyte species, amongst which at least one species shows host specificity.

Fungal symbionts resembling endophytes have also been reported from healthy lichen thalli forming persistent and symptomless infections^{11–16}. Miadlikowska *et al.*¹⁷ used the term ‘endolichenic’ fungi for endophytes isolated from lichens. These endolichenic fungi represent lineages of Ascomycota that are distinct from lichen mycobionts (the primary fungal component of the lichen thallus), lichenicolous fungi (which fruit or are otherwise symptomatic on thalli), and incidental fungi on thallus surfaces^{11,18,19}. They are known from every lichen species sampled to date at sites ranging from the Arctic to the tropics¹¹, but have been characterized in only a few communities^{11,13,14,16}.

These endolichenic fungi colonize either inter- or intracellularly and may be either localized or systemic. Microdissection demonstrates that they live in close association with photobionts and are relatively rare in the mycobiont-dominated cortices and medulla¹¹. Majority of these isolates belong to ubiquitous genera (e.g. *Acremonium*, *Alternaria*, *Cladosporium*, *Coniothyrium*, *Epicoccum*, *Fusarium*, *Geniculosporium*, *Phoma*, *Pleospora*), but some genera are common in both tropical and temperate climates (e.g. *Fusarium*, *Phomopsis*, *Phoma*), while members of the family Xylariaceae along with *Colletotrichum*, *Guignardia*, *Phyllosticta* and *Pestalotiopsis* predominate as endophytes in the tropics.

In India, studies on endolichenic fungi have been initiated recently^{16,20,21}. Suryanarayanan *et al.*¹⁶ have isolated endolichenic fungi from tropical regions of South India and reported 33 taxa along with mycelia sterilia. In contrast, Tripathi *et al.*^{20,21} worked on endolichenic fungi of temperate regions of Kumaun Himalaya and isolated seven taxa, excluding mycelia sterilia as endophytes from *Physcia dilatata* and *Heterodermia flabellata*.

This further led authors to work on endolichenic fungi of some Kumaun Himalayan macrolichens. For isolating endolichenic fungi the macrolichens were collected from different forests of Kumaun Himalaya and taken in sterile polythene bags to the laboratory and processed within 24 h of collection. For each lichen, 100 segments were randomly cut from the thallus and surface sterilized following the modified protocol of Suryanarayanan *et al.*¹⁶. The efficacy of surface sterilization was confirmed by pressing the sterilized lichen thallus segments onto the surface of PDA (potato dextrose agar) medium. The absence of growth of any fungi on the medium confirmed that the surface sterilization procedure was effective²². The samples were cultured on PDA medium supplemented with streptomycin sulphate (150 mg/l), incubated at 25°C and left for 4 weeks for sporulation. Endophytic fungal species were identified on the basis of cultural characteristics and morphology of fruiting bodies and spores using standard texts and keys^{23–29}. Cultures that failed to sporulate were recorded as