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Flower design *vs.* Pollination syndrome: A pilot screening of the Morpho-Functional traits in *Xanthophyllum arnottianum*wt. (Polygalaceae)

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Abstract

The flowers of *Xanthophyllum* highly resemble those of keeled flowers and show an inclination towards entomophily. Though cross-pollination is preferred, it is also observed that the unopened buds are viable for self-pollination. Field observations showed that the insects belonged to the families Hymenoptera and Lepidoptera visited the flowers. Lab studies showed that the stigma is receptive before anthesis and pollen tubes are found growing inside the stylar column. The present study was an attempt to understand the morphology and function of different parts of *Xanthophyllum* flowers as well as their role related to the pollination mechanism.

Keywords: endemic, floral larceny, polygalaceae, western ghats, *xanthophyllum*

Introduction

Flowers are the structures of reproduction with distinct organizations to maximize the successful establishment of an upcoming generation (Sauquet & Magallon, 2018) [13]. Though the flower structures are conserved largely across the sister groups, they indeed show some characteristic diversity in features such as flower architecture, flower advertisements, and rewards (Sauquet et al., 2017) [14]. These attributes may be correlated with the selection pressure exerted by floral mutualists and antagonists besides abiotic factors (Gervasi & Schiestl, 2017) [5]. Generally, pollination syndromes denote convergent floral adaptations to unique functional pollinator groups (Dellinger et al., 2019; Stebbins, 1970, Ashworth et al., 2015) [2, 16, 1].

The concept of pollination syndromes was much debated over the recent decades (Rosas-Guerrero et al., 2014) [1]. The tropics had the strongest associations between flower character traits and functional pollinator groups (Ashworth et al., 2015) [1]. Several specialized pollination systems have been reported from hotspots with an exceptional diversity of endemic species all over the world (Goldblatt & Manning 2000) [6]. But there is an alarming decline of pollinator populations across the globe, especially in biodiversity hotspots (Mitchell & Ashman 2008) [9]. Moreover, the occurrence of diverse endemics in those biodiversity hotspots was at a higher risk of extinction than a broadly distributed taxa (Vamosi et al. 2006) [17]. Hence, a better understanding of the inter-relation between specialized plant-pollinator system and floral adaptations are of prime importance in terms of conservation (Johnson 2004) [7]. Only a little is understood so far about the reproductive interactions of the majority of plants in species-rich tropics. Several functional or adaptive floral traits, such as self-incompatibility, dichogamy, and herkogamy, have evolved to avoid or minimize the effects of self-interference with pollination and thus improve outcrossing rates (Lloyd and Webb, 1986) [8]. To understand the evolution of floral traits, we must first identify the various elements involved in the process such as, the structures in which pollen is presented to the pollinators (Faegri and van der Pijl, 1979) and the mechanisms through which pollen is secondarily presented (Westerkamp and Weber, 1997) [18].

The present study in *Xanthophyllum arnottianum* was aimed at the understanding of flower structure, specific adaptations of the flowers for surpassing pollinator competition from the co-flowering neighborhood as well as to analyze the pollinator constancy.

Materials and Methods

Study area

X.arnottianum is an endemic understory tree of the southern Western Ghats distributed along the riparian belt of wet rainforests. They tend to populate as distinct groups in Kerala, parts of Karnataka, and Tamil Nadu. Only a few reports were found from Tamil Nadu (only in Tirunelveli district) compared to the southern districts of Karnataka. Kerala has a notable but discontinuous distribution of *X.arnottianum* records in its natural forest cover. Hence, the state is selected for the present study, and also the results could be compared better with minimum interference from external determinants. Three geographically and reproductively isolated populations of *X.arnottianum* were selected at Ponmudi, Vazhachal, and Thavinhal of Kerala state (8.7599°N –11.8488°N and 77.1169°E –75.9521°E) for the present study.

Flower morphology

Periodic field visits were carried out to study flower morphology, opening mechanism of the keel petals, and the subsequent anther release at different phenological stages. The flower morphology was observed by regular field visits during peak flowering seasons of February-April (2017-2019). The macro-morphic measurements were taken using a vernier caliper while the micro-morphic measurements were done using a stereomicroscope with the camera. A dissection microscope is used to understand the interior segments of floral parts.

Morpho-functional screening

The structure of inflorescence and flower was studied in detail with a notion to identify potential morphological traits which aid to effect pollination. Compared with other pollinator interaction mechanisms reported from different plants of related genera. The presence of visual cues such as nectar guides, color and shape of the corolla, and rewards like nectar and pollen were recorded. The number of flowers per inflorescence and their positioning on the inflorescence axis were also noticed. The mechanism of flower response to the flower visitors was observed.

Pollinator activity

Though the activity of pollinators is a random effect, the consistency of pollinator visits was identified by placing Insects nets and traps at different time intervals. The duration of flower visits was split into three sets of 8 hours per day and then observed for trapped insects. The task was repeated over three trials in all three populations with at least a two-week break between each trial per annum. The insects collected were identified using a regional insect manual. The foraging of the insects was recorded using a camera to understand the mechanism of interaction.

Results and Discussion

Flower morphology

The inflorescence is a terminal or sub-terminal or axillary panicle. The flowers were borne on a small pedicel and arranged on the inflorescence axis as a racemose panicle. Flowers are white or yellowish-green. Sepals are free, imbricate, pubescent, and five in number. Corolla is free, pentapetalous, zygomorphic, and imbricate. A characteristic hypogynous, annular, deeply lobed disk is present. Stamens are eight in number of which six fused at the base of the corolla and two attached to the basal disk. Style is pubescent, stigma papillate, and slightly bifid. The ovary is bicarpellary syncarpous with four ovules arranged in the parietal placentation.

Morpho functional traits of the flower

The flower is zygomorphic and resembles papilionaceous corolla which coincides with the generalized view on convergent evolution of species. According to Rathcke (1983) [11], community studies on sympatric species had significant evolutionary divergence or convergence and associated specialization in pollination systems. When flowers from multiple families adopt similar and generalized morphological traits, especially in their reproductive attributes related to pollination syndrome, they may be considered to evolve in the same evolutionary pathway (Dilley et al. 2000) [3]. The signaling flag petals of Papilionaceae were replaced with a pair of comparatively small petals and may be considered standard petals. 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). The presence of the keel complex serves the purpose of secondary pollen reservoir as well as a resting platform for the visiting insects. Butterflies often chose to land while visiting a flower. 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 wing petals serve as an alighting platform for insects visiting the flowers.

In *X.arnottianum,* the flowers show a range of color transitions from white to whitish green and finally to fluorescent yellow. The buds develop into white flowers at the beginning and were observed for high frequencies of visitation by moths than bees. Slowly the color changes to whitish green after complete anthesis with the moderate activity of flower visitors. The final color change to fluorescent yellow was equally recorded for high visitations from bees and butterflies. The inflorescences may be single or branched with about 5-12 mature flowers in varying colors like white, whitish-green, or fluorescent yellow. The arrangement of flowers along the inflorescence axis is of significant importance to attract many pollinators and to effect pollination. *X.arnottianum* has an infinite raceme with mature flowers at the base of the inflorescence axis and the buds were produced spirally towards the tip of the inflorescence axis in descending order of maturity. The completely opened flowers must be perfectly advertised to attract potential pollinators. The flower presentation angle in *X.arnottianum* was recorded to be 142°56′ ± 12°40′ similar orientation was reported from *Polygala vayredae* (Castro *et.al*. 2008). The flowers adapted to bee pollination often showed contrasting color guides over the corolla near the landing platform to direct them towards flower rewards. In *X.arnottianum* the standard white petals were contrasted with a bright yellow nectar guide signaling the insect towards nectar. As the flower ages, the petals turn yellow without any demarcation over them as nectar guides. This was also accompanied by the decreased nectar secretion as the flower completes blooming. This may be correlated as the pollination is over and no further need for attracting pollinators with little nectar reserve. *X.arnottianum* was noticed to produce both nectar and pollen grains in ample quantities. The nectar is produced from floral nectaries as well as extra-floral nectaries. The pollen grains were stored inside the keel complex to provide secondary pollen other than those from anthers. *X.arnottianum* has a disc beneath the ovary producing a constant supply of nectar. The nectar disc is grooved in which the gynoecium is placed in the middle. The disc has distinct wedges forming room for the temporary storage of nectar. The concentration of the nectar varies with the temperature and humidity of that particular day. Dichogamy was observed in *X.arnottianum,* i.e. the anthers mature a day before the stigma releasing the pollen grains on the stigmatic surface. The sigma shows considerable receptivity following this event and the pollen tubes emerge out and penetrate the stylar column resulting in successful bud pollination.

Pollinator activity

Insects that visited the flowers belonged to Hymenoptera and Lepidoptera. The insect visitors included *Apis dorsata, A. cerana, Trigona iridipennis, Danaus genutia, Euploea core, Moth sp.,* and *Xylocopa* sp. The carpenter bee species were noticed as nectar robbers while all others were both pollen and nectar foragers. The flowers were visited frequently by bees but the consequent visits were shorter than the first one. The insects often landed on the wing petals and the keel, exerting their weight as a thrust to the keel petal. This movement opens up the passage towards nectar over the stigma during which the insect's abdomen carrying pollen got deposited on the stigmatic surface (sternotribic pollen deposition). The carpenter bees were comparatively larger than the flowers and they simply reach out for the nectar by piercing a hole near the throat of the flower and suck out the nectar without achieving any pollination resulting in floral larceny. Similar pollinator activity was observed in *Rhynchosia beddomei* (Raju and Rao, 2016) [10].

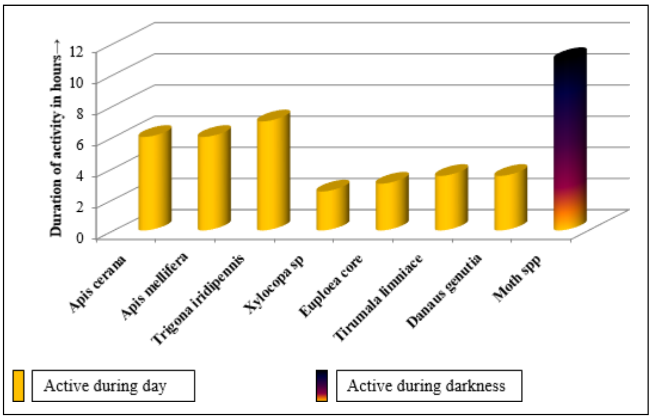

Fig 1: Pollinator activity in *X.arnottianum*

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