POLLINATION ECOLOGY OF THE ANNUAL HERB, *HEDYOTIS BRACHIATA* (RUBIACEAE)

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**Abstract** – *Hedyotis brachiata* presents a phenology that concentrates vegetative growth and reproduction during rainy and winter season. In this study, we investigated floral morphology, floral biology, pollination, pollinators, fruit and seed dispersal aspects. It is an annual herb that grows in open, sandy soils. It is distylous, herkogamous and self-compatible. The pin and thrum plants produce high natural fruit and seed set, but it is highest in thrum plants. Autonomous selfing occurs in thrum flowers while it is precluded in pin flowers, however, selfing occurs due to the foraging activity of thrips. The plant is pollinated by honey bees and lycaenid butterflies. Other insects also pollinate the plant but they are occasional foragers. The plant produces multi-seeded capsules which mature within three weeks. Fruit is a non-fleshy erect and cup-like capsule which dehisces septicidally. The seed dispersal modes include anemochory, barochory, ombrochory and hydrochory. The seeds are dormant, germinate and produce new plants seasonally. Therefore, the study substantiates that this plant is evolved to complete its entire life cycle seasonally.

**Keywords** – *Hedyotis brachiata*, distyly, herkogamy, honey bees, lycaenid butterflies, anemochory, barochory, ombrochory and hydrochory.

**Introduction**

Rubiaceae is one of the largest angiosperm families distributed mainly in the tropics and sub-tropics of the world (Eriksson & Bremer, 1991). The species of this family have remarkable ecological, economical and taxonomical importance (Perveen & Qaiser, 2007). The genus *Hedyotis* with 515 species belong to this family and distributed in the tropical and sub-tropical regions worldwide, mainly Australia, Africa, Eastern and Southeast Asia and the Americas. It is very variable and includes annual or perennial herbs, sub-shrubs, weak stragglers, weak climbers, shrubs or small trees (Vaes et al., 2006; Viswanathan & Manikandan, 2008; Tao & Taylor, 2011; Wikstrom et al., 2013). In India, there are about 75 species, many of them are restricted to the hill areas of southern India, especially the Western Ghats (Dutta & Deb, 2004). These species are important constituents of herbaceous layer in open habitats, roadsides and agricultural fields.

*Hedyotis* has a broad spectrum of breeding systems, including distyly, dioecy and herkogamy (Robbrecht, 1988; Wagner & Lorence, 1998; Ko, 1999). Pollen dimorphism occurs with respect to size, shape, and exine characteristics in some distylous species of *Hedyotis* (Naiki & Nagamasu, 2004; Castro et al., 2004). *H. nigricans* is distylous and displays heteromorphic incompatibility that precludes self- and intra-morph cross-fertilization and allows only inter-morph cross-fertilization (Ornelas et al., 2004). *H. salzmannii* is distylous, self-compatible and pollinated by bees and flies (Riveros et al., 1995). *H. acutangula* is dimorphic, distylous, cryptically self-incompatible and dominated by legitimate (inter-morph) mating (Wu et al., 2010). *H. pulcherrima* is isoplethic containing pin and thrum flowers with an equilibrium of 1:1 ratio. The two flower morphs exhibit a precise reciprocal herkogamy (Liu et al., 2012). These reports indicate that the previous workers mainly concentrated on the functionality of
sexual systems in *Hedyotis*. There is almost no information on the pollinators of individual species of this genus, at least for the species in which sexual systems have been studied. The present study is aimed at providing the information on the pollination ecology of *H. brachiata*. Since there is no basic information on any aspect of *H. brachiata*, the question of testing any hypothesis in the functional aspects of its pollination ecology does not arise. The work reported in this paper would be very valuable for taking up work in this subject on other species of *Hedyotis*.

**MATERIALS AND METHODS**

The seasonal annual herb, *Hedyotis brachiata* was selected for study during 2014-2016 in Visakhapatnam and its surroundings, Andhra Pradesh, India (17°42'N Latitude and 82°18'E Longitude). Since this plant is dimorphic and distylous, pin and thrum forms have been identified. Accordingly, all aspects included in the study for pin and thrum plants were detailed. The inflorescence type and the number of flowers per inflorescence were noted. Ten inflorescences were tagged prior to commencement of their flowering and followed daily for recording the flowering duration of the inflorescence. Twenty five fresh flowers were used to record the floral details such as flower shape, colour, odour, sex, symmetry, floral mechanism, calyx, corolla, stamens and style and stigma and ovule number. Ten inflorescences which have not initiated flowering were tagged and followed daily to record the duration of flowering, anthesis schedule and the timing of anther dehiscence. Twenty five fresh flowers were used to record the floral morphological details and the measurements were presented as mean and standard deviation. Nectar could not be measured and analyzed due to its secretion in minute quantity which was further depleted by thrips during mature bud and flower life. Twenty mature, but un-dehisced anthers were collected from ten plants and examined for pollen output as per the protocol described in Dafni et al. (2005). The calculation of pollen output per flower (X ± s.d.) and pollen-ovule ratio was done as per the formulas described in Cruden (1977). Ten flowers each from five plants were used to test stigma receptivity. It was tested with hydrogen peroxide from mature bud stage to flower closure/drop as per Dafni et al. (2005). Further, the receptivity was also observed visually whether the stigma is shiny, wet or changing colours or withering. Insects foraging at the flowers were observed from morning to evening on four different days for their mode of approach, landing, probing behavior and contact with the floral sexual organs. Bees, wasps and flies were identified with the representative specimens available with the Department of Environmental Sciences, Andhra University, Visakhapatnam. Butterflies were identified by consulting the books of Kunte (2007). The foraging visits of insects were recorded using 2 x 2 m area of flowering patch for 10 min at each hour for the entire day on four different days and the data was tabulated to record the foraging pattern and the percentage of visits made by them. The pollen/nectar collection behaviour of insects was carefully observed to assess their role in effecting pollination. Ten specimens of each insect species were captured during peak foraging period and brought to the laboratory. Each specimen was washed in ethyl alcohol, stained with aniline-blue on a glass slide and observed under microscope to count the number of pollen grains present. From this, the pollen carried by each insect species was calculated to know the pollen carryover efficiency and the same is presented as mean and standard deviation. Pin and thrum plants that have not initiated flowering were tagged and followed to record fruit and seed set rate in open-pollinations. Fruit maturation period, the fruit and seed morphological characteristics were recorded to evaluate their adaptations for dispersal by different means. Further, the seed dispersal modes were examined in the field. The aspects of seed germination and establishment of populations were observed briefly in the field.

**RESULTS**

**Flowering phenology**

The plant is an annual diffuse glabrous herb with sessile, linear-lanceolate leaves. It grows in open, sandy soils during rainy and winter season (Fig. 1a, b). The stem is green initially and purplish to dark purple later as the plant ages. Individual plants are multi-stemmed and they branch out in prostrate form carpeting the soil layer. The plants appear from the seed following the monsoonal rains in June. They grow quickly, flower from July onwards and extend up to February depending on the soil moisture status. But, flowering intensity is confined to September-November. The inflorescence is a pedunculate terminal panicle with a small cluster of flowers. The flowers are dimorphic and distylos representing the classical pin and thrum morphs. Individual plants produce a single flower morph, pin or thrum (Fig. 1c, d). The population level ratio of pin and thrum plants is 0.8:1. The inflorescences of both pin and thrum flowers produce almost the same number of flowers; their number averaged to 10.32 ± 1.98 for pin and to 9.92 ± 1.87 for thrum plant which anthesce within a week.
Flower morphology

The flowers of both pin and thrum morphs are pedicellate (0.7-1 cm long), funnel-shaped, and stand out prominently. The flowers are small, white, odourless, bisexual, actinomorphic; 5.8 ± 0.4 mm long and 4.8 ± 0.4 mm wide in pin morph and 5.5 ± 0.4 mm and 4.6 ± 0.4 mm in thrum morph. The calyx in both pin and thrum morphs consists of four 2.6 ± 0.4 mm long green, triangular and glabrous sepals which are united at the base. The corolla of both pin and thrum morphs is white with purple tinge, funnel-shaped (3.6 ± 0.4 mm long), tubular and 4-partite at the top, with oblong-linear lobes. Moniliform hairs are present on the inside of non-tubular part of the corolla (Fig. 1n). The stamens are four, white, alternate with petals (Fig. 1h) and anthers are dithecous; they are 2.4 ± 0.4 mm long and in pin morph and 3.1 ± 0.4 mm long in thrum morph (Fig. 1g). The filaments are pubescent with 1 mm long anthers in both pin and thrum morphs. The ovary of pin and thrum morphs is bicarpellary, bilocular, syncarpous with 57.72 ± 9.22 ovules in pin morph and 60.99 ± 8.71 in thrum morph (Fig. 1k, q), the ovules are arranged on sub-basal placentation; the ovary is 4.7 ± 0.4 mm long in pin morph and 2.6 ± 0.4 mm in thrum morph. The style and stigma are white and spring up from the center of the flower (Fig. 1o), it is 3.8 ± 0.4 mm long in pin morph and 2.1 ± 0.2 mm long in thrum morph (Fig. 1g). The stigma is bi-lobed, larger in thrum flowers than in pin flowers but it is completely divergent in pin morph (Fig. 1p) and partially divergent in thrum morph (Fig. 1j).

Floral biology

The flowers of pin and thrum morphs open during 0700-0900 h (Fig. 1e, f, l, m) and the anthers dehisce almost simultaneously in mature bud stage by longitudinal slits. The pollen grains of both the morphs are white, powdery, olate-spheroidal, tri-colporate, ornamented and 29.05 ± 4.15 µm in size. The pollen output per anther/flower is almost similar in
both the flower morphs and it is 1,409.6 ± 88.37 per anther and 5,638.4 ± 353.51 per flower (Fig. 1i). The pollen-ovule ratio is 82:1. The style and stigma do not contact the dehisced anthers at any stage during mature bud or flower life. The stigma is wet, shiny and receptive after anthesis and ceases it by about 1700 h of the same day. The nectar is produced in traces only. The flowers of both flower morphs close back partially by the evening of the same day. The petals, stamens, style and stigma fall off on the next day while the calyx gradually grows and bulges into fruiting calyx.

Foraging activity

Thrips were found to use the flower buds of both pin and thrum morphs for breeding. They also used flowers for pollen and nectar; while collecting the forage, they effected pollination. The flowers were indiscriminately foraged by honey bees, wasps (Hymenoptera), flies (Diptera) and butterflies (Lepidoptera) during 0800-1600 h with more activity during 0900-1100 h (Fig. 2). The honey bees were *Apis florea* (Fig. 3a, b, h, i) and *Apis cerana* (Fig. 3c). The wasp was *Scolia* sp (Fig. 3d, j). The flies were *Sarcophaga* sp. (Fig. 3, l) and an unidentified species (Fig. 3k). The butterflies were *Acraea violae* (Fig. 3, e, m), *Zizeeria karsandra* (Fig. 3f, n), *Freyeria trochylus* (Fig. 3g), *Spindasis vulcanus* and *Chilades pandava*. Of these, honeybees foraged for both pollen and nectar while all others foraged for nectar only. Honeybees and lycaenid butterflies were the regular foragers while all other insects were occasional foragers. Of the total foraging visits of insects, bee visits accounted for 35%, wasps 14%, flies 19% and butterflies 32% (Fig. 4). The honeybees and lycaenid butterflies being regular foragers made multiple visits to the same flowers/inflorescences of different conspecific pin and thrum plants in quest of more forage effecting both self and cross pollination within and between dimorphic plants. The occasional foragers also visited both pin and thrum plants to collect the nectar since the latter is produced in traces only and it is mostly consumed by thrips and then by regular foragers. While probing and collecting the forage from the flowers, they contacted the stigma first and then the stamens situated far below in pin flowers, and the stamens first and then the stigma situated far below in thrum flowers. The body washings of these insects from pin and thrum flowers revealed that all of them carry pollen to different extents - honey bees carried highest number of pollen grains than all other insects (Table 1). Fruit set in pin flowers vector-dependent and while in thrum flowers, it is a function of autogamy and entomophily.

Fruiting behaviour

The pin and thrum flowers produce fruits within three weeks; the natural fruit set rate is 80.42% in pin plants and 98.79% in thrum plants. The inflorescence level fruit set rate is 8.3% in pin plants and 9.8% in thrum plants. Fruit is a non-fleshy capsule, sub-globose, somewhat dicoccous, membranous and glabrous (Fig. 3o, p). The seed set rate per fruit is 47.11± 6.24 in pin plants and 49.69 ± 6.56 in thrum plants. Mature
and dry fruits dehisce septicidally into two valves releasing seeds into the air, which are then carried away by wind and also by gravity. Further, the seeds also eject by the falling of water drops into the capsule during rainfall and then they are dispersed by flowing water. The seeds are brownish yellow, tiny, 3-ridged shining smooth, testa reticulate, 0.2 x 1 mm (Fig. 3 q). Seeds are dormant and germinate only during rainy season to produce new plants.

**Discussion**

The genus *Hedyotis* has a broad spectrum of breeding systems, including distyly, dioecy and herkogamy (Robbrecht, 1988; Wagner & Lorence, 1998; Ko, 1999). This study shows that *H. brachiata* is distylous, herkogamous and self-compatible. Distyly is a function of the slowdown of stigma elongation in thrum flowers and the elongation of stigma at constant rate in pin flowers. This form of growth pattern in the stigma has been reported in *Hedyotis caerulea*, *H. salzmanii* (Riveros et al., 1995), *Guettarda scabra* (Richards & Koptur, 1993), *Psychotria chiapensis*, *P. poeppigiana* and *Bouvardia ternifolia* (Faivre, 2000). Distyly in *H. brachiata*...
is accompanied by differences in flower length, the number of ovules, ovary length, stigma lobe length and the extent of divergence of stigmatic lobes. The flowers of pin morphs are larger than that of thrum flowers. The number of ovules in pin flowers is slightly more than that in thrum flowers. The ovary of thrum flowers is smaller than that of pin flowers. The stigma lobes of thrum flowers are larger than those in the pin flowers. The pollen grains are isomorphic, tricolporate and display the same characters in both the flower morphs. This is inconsistent with the hypothesis that there is a trade-off between size and the number of pollen grains (Cruden & Lyon, 1985; Richards, 1997). With isomorphic pollen characters and similar output per flower, the pin and thrum plants produce highest natural fruit set suggesting that self-compatibility is largely functional. However, the variation in fruit set rates between pin and thrum plants is quite significant, it is highest in thrum plants. Further, seed set rate is also the highest in thrum flowers compared to that in pin flowers. This situation is indicative of the occurrence of autonomous selfing in thrum flowers while it is precluded in pin flowers by the elongation of stigma far beyond the anthers. In thrum flowers, autonomous selfing occurs due to the falling of pollen by gravity from the anthers to the stigma which is situated far below the anthers. The larger stigmatic lobes consisting of papillae appear to be an evolved character to capture the falling pollen grains efficiently due to which selfing occurs without the mediation of any vector. Further, weak protandry largely facilitates the occurrence of selfing or cross-pollination in the presence or absence of pollen vectors. In pin flowers, autonomous selfing is not possible but the foraging activity of thrips contribute to selfing due to weak protandry. The short-lived flowers further substantiate that the plant is primarily self-pollinating while keeping the options open for intra- and inter-morph cross-pollination through distyly. Rubiaceae presents a wide range of floral visitors due to the presence of a wide range of flower forms, sizes and colours. Most of these pollinators include insects while birds and bats play a minor role in pollination. Among insects also, bees are important pollinators especially for small-flowered species; the showy large-flowered species are adapted for pollination by butterflies and hawk-moths. The butterflies are pollinators for scentless flowers while hawkmoths are pollinators for long-tubed fragrant flowers (Puff et al., 2005; Consolaro et al., 2005). In the present study, Hedyotis brachiata is consistently pollinated by honey bees and lycaenid butterflies. Other insects also pollinate the plant but they are occasional foragers. Thrips use the flower buds for breeding and cause pollination during flower life by moving within and between flowers/inflorescences of the same or different individuals of the pin as well as thrum plants for pollen and nectar collection. Their foraging activity appears to be ensuring the occurrence of natural fruit set to a large extent both in pin and thrum plants. Further, these thrips deplete the nectar which is secreted in traces and this is compelling the regular as well as occasional foragers to visit the same flowers repeatedly both in pin and thrum plants, resulting in the promotion of intra- and inter-morph self- and cross-pollinations. Such a foraging activity is collectively contributing to the enhanced levels of natural fruit and seed set. The self-compatibility, weak protandry, autonomous selfing (in thrum flowers), foraging activity of thrips, regular and occasional pollinators are

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<tr>
<th>Insect species</th>
<th>Sample size (N)</th>
<th>Number of pollen grains</th>
<th>S.D</th>
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<tr>
<td></td>
<td></td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Apis cerana</td>
<td>10</td>
<td>54 - 209</td>
<td>115.5</td>
</tr>
<tr>
<td>Apis florea</td>
<td>10</td>
<td>67 - 302</td>
<td>162.1</td>
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<tr>
<td>Scolia sp.</td>
<td>10</td>
<td>24 - 71</td>
<td>47.7</td>
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<tr>
<td>Sarcophaga sp.</td>
<td>10</td>
<td>21 – 48</td>
<td>36.1</td>
</tr>
<tr>
<td>Unidentified fly</td>
<td>10</td>
<td>13 – 54</td>
<td>32.5</td>
</tr>
<tr>
<td>Acrea violae</td>
<td>10</td>
<td>24-78</td>
<td>48.5</td>
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<tr>
<td>Zizeeria karsandra</td>
<td>10</td>
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<td>40.7</td>
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<td>10</td>
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<td>Spindasis vulcanus</td>
<td>10</td>
<td>23-64</td>
<td>42.1</td>
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Table 1. Pollen recorded in the body washings of insects collected on pin and thrum flowers of Hedyotis brachiata.
collectively producing more than 90% of fruit set but only nearly half the number of ovules produce seeds. The seed set rate recorded in this species indicate that all the ovules do not produce seeds, which could be due to unfertilized ovules, the selective abortion of low quality seeds arising from the self-pollination and the state of nutrient environment in the soil. However, the extended period of flowering over two successive seasons from wet to winter season is advantageous for the plant to optimize seed set rate and grow as a colonizer during wet season of the following year.

Puff et al. (2005) reported that fruits of Rubiaceae are of capsule type and classified into three types: those that split open at maturity, those that break into one-seeded mericarps and those that remain indehiscent. The dehiscent capsule types expose and release the seeds. The seeds disperse by four different means, anemochory, ombrochory, ornithochory and hydrochory. Anemochory is very widespread while ornithochory is the most prevailing one. Ombrochory is uncommon and it occurs in herbaceous taxa with erect, cup-like capsules. Hydrochory involving sea currents occurs in genera like Guettarda and Scyphiphora. In the present study, Hedyotis brachiata produces multi-seeded capsules which mature within three weeks. Fruit is a non-fleshy erect and cup-like capsule; the fruits are septicidal with persistent calyx and dehisce from the top to release seeds into the air. Further, the seeds also disperse by gravity, by water drops falling into the dry capsule and by run-off during rains. Therefore, H. brachiata exhibits anemochory, barochory, ombrochory and hydrochory; these modes collectively enable H. brachiata to be a colonizer species, especially in open sandy soils. This study indicates that this species appears to have developed adaptations to grow in sandy soils, maximize seed production using local honey bees and lycaenid butterflies each year and subsequently to colonize the favourable areas during growth season. Individual plants flower for a few weeks but they appear in flowering at population level for two seasons, wet and winter season. This situation appears to be a function of fixed period of dormancy for individual seeds, and accordingly, the seeds produced early would germinate early while those produced late would germinate late, in effect, the vegetative growth and flowering is extended until the onset of dry season.

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**References**


