

Pollination ecology of the Red Sanders *Pterocarpus santalinus* (Fabaceae), an endemic and endangered tree species

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***Pterocarpus santalinus* is an endemic and endangered tree species in the Eastern Ghats of India. It shows flowering during dry season. The flowers are large, yellow, bisexual and papilionaceous. They open at around midnight and offer pollen and nectar for the flower visitors which include only honey bees, *Apis dorsata*, *A. cerana indica* and *A. florea*. *A. dorsata* was the main pollinator and made visits soon after anthesis under moonlight and disappeared at 0730 h. The other two species made visits only during early morning hours. This tree species shows facultative xenogamy, but mostly eliminates growing fruits from self-pollinated flowers. The natural fruit set rate was 6% only and this low fruiting rate is attributed to different factors.**

PTEROCARPUS is a genus of trees and woody climbers distributed in the tropics throughout the world. There are only four species in India. Among these, *P. dalbergioides*, an evergreen tree occurs only in the Andamans. *P. indicus*, a native of Malaysia, has been introduced and planted as a garden and avenue tree in the Andamans, West Bengal, Tamil Nadu and Maharashtra. *P. marsupium* is a deciduous tree and occurs commonly in hilly regions throughout the Deccan Peninsula and extends to Gujarat, Madhya Pradesh, Uttar Pradesh, Bihar and Orissa¹. *P. santalinus* is also a deciduous tree and is restricted to Cuddapah and Kurnool districts in Andhra Pradesh and Arcot and Chingelpet districts in Tamil Nadu up to 500 m (ref. 2). It has been reported to be a native of Africa³, but its entry into a restricted part of India remains a mystery. All four species are valued for the wood. Except *P. marsupium*, the other three species are valued for a red pigment, santalin. However, only *P. santalinus* is highly valued for its heavy, dark claret-red heartwood which yields 16% of red colouring matter to santalin¹. In recent years, a variant in this species which has wavy-grained wood has leapt into sudden prominence because it is highly valued in the export market. Trees with this variant character are rare in nature and they seem to show no apparent morphological differences to differentiate them from the normal-grained trees. It is unclear how this character has arisen⁴.

The natural habitat of *P. santalinus* is a hilly region with hot dry climate. It is a strong light demander and

does not tolerate overhead shade. It cannot withstand water-logged conditions. It is listed out as an endemic taxon of the Eastern Ghats because of its restricted occurrence². Further, it is now considered to be endangered because its natural habitat is constantly subjected to human pressure. Roubik³ indexed that there is no information on breeding systems and pollinators of this species. This information is essential for any effort to conserve and improve this species, especially to increase the frequency of occurrence of trees with wavy-grained wood through cross-pollination to a maximum extent among the different superior clones⁴. Since there is no information on the pollination ecology and breeding systems of this species, the present study was carried out to investigate the same. The results are discussed in detail.

The natural populations of *P. santalinus* Linn. f. distributed in the natural regeneration plot of 22 ha of Sri Lankamalai Reserve Forest, 8 km away from Siddavatam towards Badvel, Cuddapah District (longitude 78°58'E and latitude 14°28'N) were used for the present study. This region comprises dry deciduous forest mixed with thorny plant species and is subjected to grazing and burning. Flowering phenology was observed at plant and inflorescence level with reference to the day-to-day flowering pattern. For the latter, 50 inflorescences, selected at random from different conspecific trees were tagged before the initiation of flowering. These were followed daily and the number of open flowers was recorded. The open flowers were then removed to avoid recounting on the next day. The tagged inflorescences were followed until they ceased flowering. Fifty flowers were sampled to record floral aspects and pollen characters. The time of daily anthesis, anther dehiscence and nectar production was recorded. Pollen grain number/anther/flower was determined from 30 flowers distributed over different trees, following the procedure in Aluri and Subba Reddi⁵. Stigma receptivity was tested through hand-pollinations according to the procedure in Aluri and Subba Reddi⁵. Breeding behaviour by autogamy, geitonogamy and xenogamy was tested through controlled pollinations following the procedure in Aluri and Subba Reddi⁵. One hundred inflorescences on different trees were tagged and were followed until fruit development to observe the rate of natural fruit set. Insects (honey bees) were observed when they were seen foraging for pollen and nectar from the flowers. They were observed with binoculars and were also photographed. Flower handling behaviour was observed to determine the role of honey bees as pollinators. Visitation pattern was determined by recording the time and frequency of honey bees. The foraging activity of honey bees on moonlit nights was observed using a torchlight and headlights of a jeep. The duration of the foraging visit was taken as the time from the moment the honey bee alighted on the flower to the time it left the flower. Ten such observations were made and the mean length of a visit in seconds was calculated. The number

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of flowers visited by the honey bees was counted and the total time spent to cover these flowers was recorded with a stopwatch. From these data, the mean number of flowers visited in a minute's time was calculated. For pollen-carrying capacity, the honey bees were collected, their bodies washed (except pollen loads) with alcohol on a glass slide and stained with lacto-phenol aniline-blue according to the procedure by Dafni⁶. Ten individuals were collected for each honey bee species and washings were carried out separately. The slides were then observed under microscope and the number of pollen grains present was counted. From these data, the mean number of pollen grains adhering to the bodies of honey bees was calculated.

The flowering occurs from late March to late May at population level. Individual trees flower intermittently for about three weeks only. Within this period, mass flowering is evidenced on certain days, but there is a gap between two mass flowering days (Figure 1). The inflorescence is a simple raceme and consists of 25 ± 4 flowers (R 18–31) which anthesse intermittently over a period of 15 days. The flowers are pedicellate, bright yellow, 16 mm long, typically papilionaceous, zygomorphic, bisexual and mildly odoriferous. The calyx is tubular at the base and free towards the apex. The corolla consists of one standard petal, two winged petals and two keel petals. The stamens are ten, united into two bundles of five each and the anthers are ditheous. The ovary consists of two ovules only. In bud stage, the standard petal encloses the wing and keel petals which in turn enclose the sex organs. As the bud matures, the standard petal gradually bulges, and protrudes from the calyx. At

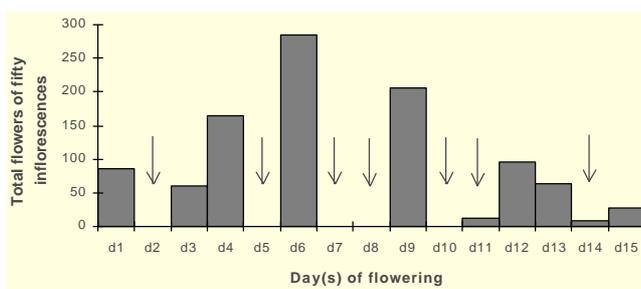


Figure 1. Day-to-day flower production of *P. santalinus*. Arrows indicate total absence or very less flowering.

anthesis which takes place around 0030 h, it unfolds exposing the wing and keel petals in which the sex organs are still enclosed. The flowers secrete nectar in trace amount. The anthers dehiscence asynchronously after anthesis; two anthers an hour later, three anthers again after one hour and the other five again after one and half hours. Their dehiscence occurs by longitudinal slits. The pollen grains are fertile, yellow, spherical, tricolpate, smooth-walled and $21 \mu\text{m}$ in size. The mean number of pollen grains per anther is 3480 and per flower is 34,800. The ratio of pollen grain to ovule number is 17,400 : 1. The stigma becomes receptive by about the time the first two anthers dehiscence and remain so until late evening of the same day. The hand-pollination tests for stigma receptivity showed 84% fruit set with fresh stigmas and 8% with 18-h-old stigmas. The hand-pollination tests for breeding systems indicated 24% fruit set and 50% seed set through autogamy, 68% fruit set and 50% seed set through geitonogamy, and 84% fruit set and 57% seed set through xenogamy. Of the two ovules per flower, only one ovule produced seed in all the modes of pollination but rarely both ovules produced seeds in xenogamous fruits. Most of the autogamous and geitonogamous fruits dropped-off prematurely, while all xenogamous fruits were retained to maturity (Table 1). The natural fruit set was 6%. A sample of 100 inflorescences consisting of 2646 flowers with 5292 ovules selected at random on different trees at flower age was used for estimating fruit set, seed set and fecundity. Among these, 170 flowers with 340 ovules set fruits with 178 seeds. Seed set was 52%. Fecundity was 3%, which was expressed in terms of total number of seeds produced against the total number of ovules in sampled inflorescences.

The flowers were exclusively foraged by honey bees comprising *Apis dorsata*, *A. cerana indica* and *A. florea* (Figure 2 a–c). Among these, the first was the most dominant in visits and number, and visited soon after anthesis on moonlit nights, showing brisk activity up to 0600 h. Later, its activity declined and disappeared at around 0730 h. In the same habitat, *Bauhinia racemosa* was the co-flowering tree and it anthesed during 1530–1830 h. The rock bee gathered pollen from this plant during moonlit hours alternately. The other two honey bee species visited the flowers during 0530–0740 h only. They were relatively less in number and visits.

Table 1. Results of breeding systems in *P. santalinus*

Treatment	No. of flowers pollinated	No. of flowers set fruit	Fruit set (%)	No. of seeds produced	Seed set (%)	No. of fruits dropped-off prematurely	Fruit drop (%)
Apomixis	50	0	0	0	0	0	0
Autogamy							
Unmanipulated	50	0	0	0	0	0	0
Manipulated	50	12	24	12	50	10	83
Geitonogamy	50	34	68	34	50	20	59
Xenogamy	50	42	84	48	57	0	0

The open flowers offer both pollen and nectar. The stigma is slightly curved upwards and extended beyond the length of the stamens. Both sex organs are enclosed by the wing and keel petals in open flowers. The honey bees collected both pollen and nectar indiscriminately. They approached the flowers in upright position, alighted on the wing and keel petals, and probed for nectar and/or pollen. While alighting on the flower, they first contacted the stigma and then the stamens with their underside, releasing them from the wing and keel petals. In effect, the bees picked up pollen on their underside and this pollen gets transferred to other conspecific stigmas in their subsequent visits. On mass blooming days, the honey bees mostly concentrated on the same tree which they first visited for collecting floral rewards while on days of little flowering, they frequently visited the flowers of conspecific trees for want of floral rewards. *A. dorsata* was efficient in harvesting floral rewards by spending very little time per flower. The other two honey bee species were relatively inefficient in depleting floral rewards as they spent more time per flower (Table 2). The body washings of honey bees for pollen grains indicated that *A. dorsata* carried more pollen than the other two honey bee species (Table 2). The difference between mean number of pollen grains carried by *A. dorsata* and *A. cerana indica* is just significant and that between *A. dorsata* and *A. florea* is highly significant. Therefore, all the three honey bee species were pollen carriers and effected pollination. However, *A. dorsata* was the main pollinator, especially for cross-pollination because of its frequent inter-tree movements.

Most tropical tree species that flower in the dry season are mass blooming^{7,8}. *P. santalinus* is no exception. But, it does not produce mass bloom daily during the flowering season, and mass bloom recurs only on certain days with a more or less definite period of interval. Such intermittent mass blooming appears to be unique, as no such pattern is found in the description of flowering patterns of

tropical plants reviewed and discussed by Bawa⁹. This flowering pattern appears to be a consequence of dry arid climate, with temperatures reaching more than 45°C and resulting in severe water stress. It is likely that water and also energy are conserved in the period between mass blooms, and invested to produce the subsequent mass bloom, and so on.

P. santalinus fruits through autogamy, geitonogamy and xenogamy, indicating that it has facultative xenogamous breeding system. Autogamy does not take place without the pollen mediation by the flower visitors. This is because the automatic contact between the anthers and the stigma within the same flower does not take place as the stigma is placed well beyond the anthers. The same is realized in the hand-pollination test for unmanipulated autogamy. Therefore, both self-and cross-pollinations depend on pollen mediation performed by the flower-visitors. Although this species is both self-and cross-pollinating, it sheds most of the autogamous and geitonogamous fruits, while retaining all xenogamous fruits to maturity. It suggests that *P. santalinus*, by predominantly cross-pollinating, leaves open the possibilities for self-pollination. This tree species might be selectively eliminating the growing self-pollinated offspring in order to allocate resources for the xenogamous fruits. Cruden¹⁰ predicted that pollen-ovule ratios are the indicators of breeding systems, and further estimated the pollen-ovule ratio for each breeding system. The pollen-ovule ratio found in *P. santalinus* is much more than that predicted for facultative xenogamy. This high pollen-ovule ratio appears to be imperative for the success of facultative xenogamy, especially for xenogamy.

Faegri and van der Pijl¹¹ gave a detailed outline of the characters of honey bee flowers. They are strongly zygomorphic, mechanically strong with landing surfaces, and are frequently intricate and semi-closed. Odours are fresh but not exceptionally strong. Nectar, produced in moderate quantities, is hidden though not very deeply.

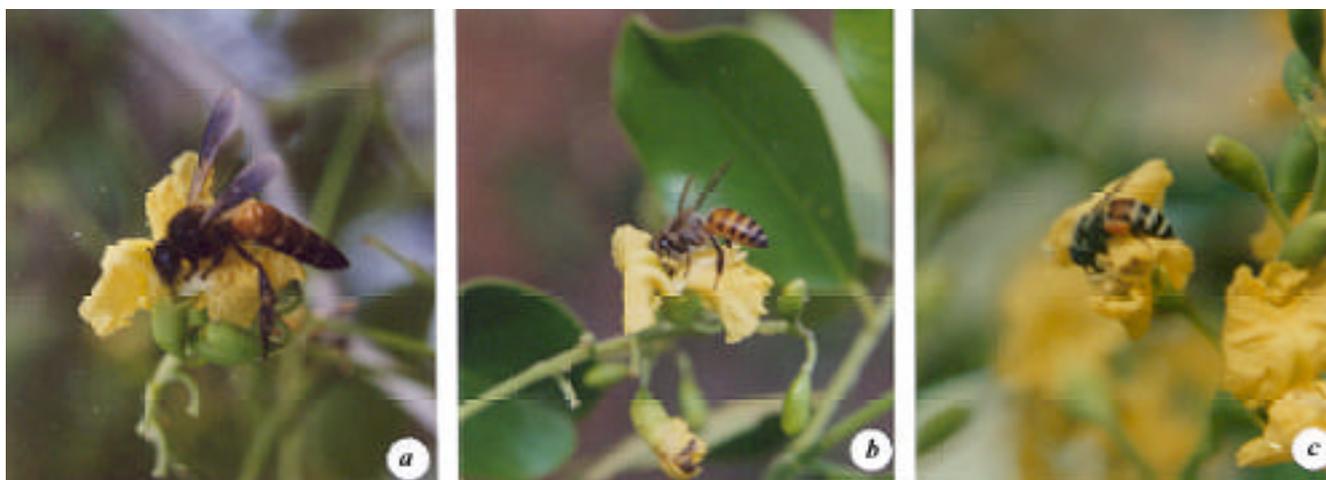


Figure 2. Pollinator bees of *P. santalinus*. *a*, *Apis dorsata*; *b*, *A. cerana indica*; and *c*, *A. florea*.

Table 2. Foraging efficiency and pollen pick-up by honeybees on *P. santalinus*

Visitor species	No. of flowers visited				Length of a visit				Pollen grains found in body washings			
	<i>N</i>	<i>R</i>	\bar{X}	SD	<i>N</i>	<i>R</i>	\bar{X}	SD	<i>N</i>	<i>R</i>	\bar{X}	SD
<i>Apis dorsata</i>	10	12–18	15	2.56	10	1–6	3	1.4	10	850–1860	1346	365
<i>A. cerana indica</i>	10	4–14	8	3.17	10	2–13	6	3.0	10	472–1420	914	530
<i>A. florea</i>	10	6–12	9	1.92	10	4–15	7	3.9	10	340–985	726	240

N, No. of insects observed; *R*, Range; \bar{X} , Mean; SD, Standard deviation.

Sexual organs are concealed, with a few stamens. They further cautioned that these characters need not be absolute, as honey bees usually visit any blossom type if rewarding. Most of these characters are found in the flowers of *P. santalinus*, and the flowers were visited exclusively by honey bees. They alight on the wing and keel petals and probe for nectar located at the flower base. Then, the force exerted by the bees causes the upper edge of the keel to rip open, and the sex organs rush out contacting the ventral side of the bees. The consequence is that the pollen is deposited on and received from the visitor, sternotribically. The forage collection by all the three honey bee species at a time on the same tree during early morning hours may cause aggressive interactions among individuals of the same or different species. In consequence, they are most likely to make inter-tree movements, effecting more cross-pollination. Further, the successive dehiscence of anthers in a flower provides an opportunity for honey bees to make multiple visits and thereby increasing the chances for the occurrence of more outcrossing^{12–14}. Among honey bees, *A. dorsata* is highly efficient in harvesting floral rewards when compared to the other two species. It flies rapidly from flower to flower, and makes frequent inter-plant movements naturally. Further, it does long hours of foraging. All this suggests that *A. dorsata* is the principle pollinator and is also very important for cross-pollination. The other honey bees with a very brief period of forage collection during morning hours, appear to be important mostly for self-pollination.

Janzen⁷ reports that dry season favours pollinating activity, while Proctor and Yeo¹⁵ state that excessively high temperatures often lead to a scarcity of pollinating insects. In the present study, the natural habitat of *P. santalinus* is noted for excessively high temperatures during dry season and also, there is a scarcity of pollinators. The pollinators were represented by honey bees only. Among these, *A. dorsata* exhibited nocturnal foraging on moonlit nights and extended foraging activity up to 0730 h. The other honey bees collected forage only during early morning hours. There was no insect activity thereafter throughout the day. This suggests that *A. dorsata* is capable of collecting forage during moonlight hours. Perhaps, this bee is intolerant to excessively high temperatures, and thus developed adaptations to forage

during night under moonlight. This finding substantiates the observation made by Dyer¹⁶ that *A. dorsata* makes nocturnal flights by using the moon's illumination; it uses moonlight to see the landmarks by which to determine the sun's position to make nocturnal flights. Sihag¹⁷ reported *A. dorsata* activity during moonlit nights on the flowers of *Albizia lebbek* in May–June when day temperature would be around 40°C. Hedegart¹⁸ found that the insect pollinators on *Tectona grandis* were unable to work at the very high temperatures. The nocturnal foraging activity of *A. dorsata* on *P. santalinus* might be to avoid excessively high temperature during daytime. The other honey bees too, restricted their foraging schedules to the early morning time. Therefore, the pollinator activity is related to the ambient temperatures, the activity being limited by low temperatures in temperate climates and by high temperatures in tropical climates¹⁹.

In *P. santalinus*, the natural fruit set is very low when compared to very high flower production. Different factors could affect low fruit set. First, this tree being able to fruit through both self- and cross-pollination, initiates more fruit production, but gradually and selectively eliminates the growing poor offspring, especially those resulting from self-pollination. The compatibility to self-pollen seems to provide fertility assurance in the event of failure of outcrossing²⁰. Secondly, this species is expected to maintain lower levels of variation because of its endemic status with restricted population size, and consequently its reduced opportunities for outcrossing²¹. Further, it has been considered to be endangered due to constant human or other pressures, and any reduction in the population size is bound to enforce inbreeding and genetic bottlenecks²¹. Thirdly, the low maternal energy investment is available during dry period for the rapidly growing offspring. These factors might be collectively responsible for the low natural fruit-set rate.

The pollination ecology of *P. santalinus* is vulnerable to dry and hot conditions. The flowers remain unvisited day-long because of the absence of pollinator activity. The natural fruit set is a consequence of pollinator activity during moonlit night and early morning hours. The self-compatibility through geitonogamy is virtually inevitable for *P. santalinus* to produce fruits in situations when pollinators are scarce. Most of the endemic and endangered plant species have been reported to be self-

compatible through geitonogamy and this selfing ability is expected to be a 'fail-safe' strategy to produce fruits when pollinators are scarce²²⁻²⁴. In *P. santalinus* too, geitonogamy exists; but it is mainly cross-pollinating, indicating that it exhibits facultative xenogamous breeding system. This breeding system is advantageous for *P. santalinus* as an endemic and endangered species, ensuring continued survival.

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MEETINGS/SYMPOSIA/SEMINARS

Skill Development Course in Bioinformatics

Date: 18-23 November 2002
Place: Madurai

Topics include: Information acquirement, storage, retrieval and transmission in biological and computer systems; Informational Biomolecules and Biochips; Signal transduction, positive and negative controls networking and crosstalk; Molecular modelling and drug design; Genomics: gene transfer and gene manipulations; Database, data mining, gene bank and blast search.

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The Second Asian Particle Technology Symposium (APT 2003)

Date: 17-19 December 2003
Place: Penang, Malaysia

Topics include: Particle and bulk powder characterization; Particle design, new technologies and industrial applications; Powder handling and multiphase flow; Solid-liquid separation process.

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IX Ranbaxy Science Foundation Symposium on Therapeutic Antibodies

Date: 3 January 2003
Place: New Delhi, India

This symposium will review the use of humanized recombinant monoclonal antibodies as new and safe drugs for therapy of rheumatoid arthritis, non-Hodgkin leukaemia, prevention of allograft rejection, autoimmune diseases and allergies. Work being conducted in India on humanized antibodies against hCG and LHRH for control of fertility and cancers, species-specific snake antivenoms, recombinant antibodies against rabies and anti IgE antibodies for allergies will also be presented. Special lectures on production of humanized antibodies and molecular pharming of antibodies will be given by experts.

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