Rarity analysis of an endangered tropical tree species of the Western Ghats, India

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The rarity of *Atuna indica*, an endemic and endangered tropical tree species of the Western Ghats, India has been analysed in this study. The phenology, floral biology, including pollen–ovule ratio, pollinators, fruit development and biotic interactions in natural dynamics were studied. Extreme reduction in the number of flowering individuals, microhabitat conditions, low fruit set, seed infestation and fruit predation were identified as the factors leading to rarity of the species *in situ*.

Keywords: *Atuna indica*, endangered species, phenological changes, rarity analysis, reproductive biology.

THIS study aims to document the reproductive biology as well as phenological changes in *Atuna indica*, an endangered tree species of the Western Ghats, India. Studies on the reproductive biology of threatened tree species provide insights into their reduced fitness/population size. For example, *Elaeocarpus blascoi*, an endangered species reported with low seedling recruitment, was found with fungal attacks on seeds (*Fusarium* sp., *Lasiodiplodia* sp. and *Pencilium* sp.)[1]. Studies conducted on the endangered *Elaeocarpus gaussenii* and *Elaeocarpus recurvatus* reported that fruit damage caused by Malabar giant squirrel and lion-tailed macaque is one of the reproductive constraints[2]. In *Talbotiella gentii*, a critically endangered tree, the stigmatic surface was found infected by fungi, thus reducing its reproductive potential[3]. Even the Evans *et al.*[4] reported reproductive constraints in endangered perennial herbaceous species such as *Eryngium cuneifolium*, *Hypericum cumulicola* and *Liatris ohlingerae.*

The present study incorporates climate data (average precipitation and temperature in Kerala, India) to discuss the phenological changes of *A. indica*. Kerala experiences different but almost stable climatic seasons such as the southwest monsoon, northeast monsoon, winter and summer. So phenological studies, including reproductive biology, will highlight the influence of climate on the reproductive performance and survival of species. The variations in atmospheric temperature, rainfall and difference in day length, etc. could signal flushing, flower initiation, etc. These changes may lead to a cascade of positive or negative influences on the depending fauna and eventually on the survival of the plant species, as the plant reproductive cycle depends on pollinators, parasites and pests, which are obligatory and species specific in nature.

Reduced reproductive potential is considered one of the driving forces towards extinction. High reproductive potential may increase seedling recruitment, subsequently resulting in flourishing of the population[5,6]. The reasons for rarity may vary from one species to another. The mode of pollination and type of dispersal impact the future population by influencing the genetic as well as the physical constitution of a population. Abnormalities in these events may result in rarity of the species *in situ*. The factors leading to species decline include reduced pollinator-driven low fruit set, self-pollination driven inbreeding depression[7], and loss of genetic variability as a compound effect. Documenting the reproductive biology of endangered plant species could help unravel their constraints. The rarity analysis of *A. indica* with an emphasis on its reproductive biology will be useful for government and non-governmental organizations in their conservation efforts of endangered tree species.

A. indica is an endemic and endangered tropical tree species of the Western Ghats, India[8]. It is distributed in the evergreen forests in the 500–800 m altitude range and grows up to 15 m height. Flowers are bisexual and cream-coloured. Slopes adjacent to water courses are the microhabitat preference of this species. Umbelliferin (an anticancer drug) has been isolated from *A. indica*[9].

Materials and methods

The population located adjacent to Kakkayam dam site, Kozhikode district, Kerala was chosen for this study (Figure 1). Monitoring and recording of flowering phenology, viz. flower-bud initiation, development, anthesis, stigma receptivity, pollen viability, pollen–ovule ratio, pollination, pollinators, blooming period, pest incidence and fruit set, was done. The data were represented as the average value of each trial[10–14].

Reproductive phenology

Data on reproductive phenology with respect to the number of inflorescences per branch, number of flowers per inflorescence, flower/inflorescence development, blooming

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period, fruit initiation and development were recorded daily. Five inflorescences per branch were tagged and monitored for flower development from bud to full bloom. The average number of days taken for each bud to bloom was calculated and recorded. The monthly mean temperature and mean precipitation of the respective area were obtained from WorldClim database using DIVA GIS.

Pollen viability

Pollen grains from fully mature flower buds were dusted into a cavity slide containing acetocarmine solution and kept for 1 h. Later observed under a compound microscope. The pollen grains stained were treated as viable and the others as non-viable. Viability test was carried out in 2 h intervals.

Pollen germination

Pollen grains from fully mature flower buds were transferred to a cavity slide containing a germination medium (sucrose 10%). Pollen germination was counted after 1 h using a compound microscope. The pollen grains with tubes longer than the diameter were considered germinated. The experiment was repeated in 2 h intervals from anthesis.

Stigma receptivity

Both physical (through hand lens) and chemical (using hydrogen peroxide) tests were conducted. In the former method, stigma with wetness, turgidity and oily nature was considered as receptive and the rest as non-receptive. In the latter method, a drop of hydrogen peroxide was added to the stigma of freshly opened flowers and the effervescence resulting from the peroxidase enzyme activity was observed for the duration of stigma receptivity[15].

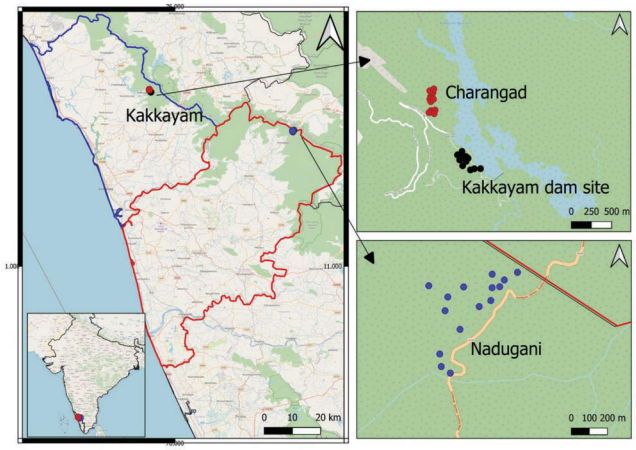

Figure 1. Distribution of *Atuna indica* in the Western Ghats, India. Study conducted on the Kakkayam dam site population.

Pollen–ovule ratio

The number of pollen grains in anthers per flower was counted using a haemocytometer[16]. The number of ovules per ovary was counted by taking sections of the ovary[17]. The pollen–ovule ratio was calculated as follows

$$\text{Pollen–ovule ratio} = \frac{\text{Pollen count per anther} \times \text{No. of anthers per flower}}{\text{No. of ovules per flower}}.$$

Pollination and insect interaction

Bagging experiments were carried out to understand the mode of pollination. Physical observations were made throughout the flowering period and insect interactions were recording during day and night hours. The taxonomic identification of insects was made using the available literature and with the help of experts.

Fruit phenology

Fruit phenology was monitored and recorded, viz. fruiting primordia, period of development, including premature abscission and pest incidence.

Results

Flowering was observed along with flushing during October–December. Fruit development started in January and fruits matured in April. The trees displayed a vegetative phase from May to September. Only one out of 89 trees in the population showed flowering. A total of 13 branches showed flowering, which included 113 inflorescences bearing 521 flowers.

Reproductive phenology

Pale green-coloured flower buds were recorded during the first week of October and mass blooming was noted after two weeks in 2017 (trees showed differential flowering in branches: in 2018, the northeastern, sun-facing branch flowered first, and 2–3 weeks later, the opposite branches flowered). Flower opening started from 0600 to 0615 h and opened fully by 0915 h. Anther dehisced through vertical slit from 0900 to 0930 h. Stigma was receptive prior to anther dehiscence (0800–0830 h, protogynous condition).

Pollen viability and stigma receptivity

Fresh pollen grains (on anthesis – 0615 h) showed 98.32% viability and gradual reduction was noticed to 93.6%,

Figure 2. ***a***, Flower buds; ***b***, flower; ***c***, fruits; ***d***, pollen grain; ***e–g***, SEM image of mouth parts of humming bird hawk moth showing pollen grains; ***h–j***, SEM image of mouth parts of *Xylocopa* sp.; ***k***, *Badamia* sp.; ***l***, *Apies mellifera*; ***m***, *Eurema* sp.; ***n***, *Graphium* sp.; ***o***, unidentified; ***p***, purple throated sunbird; ***q***, *Indrella ampula*; ***r***, pierced flower bud; ***s***, Pyralid larvae in flower; ***t***, flower bud eaten by snail; ***u***, Pyralid larvae in fruit.

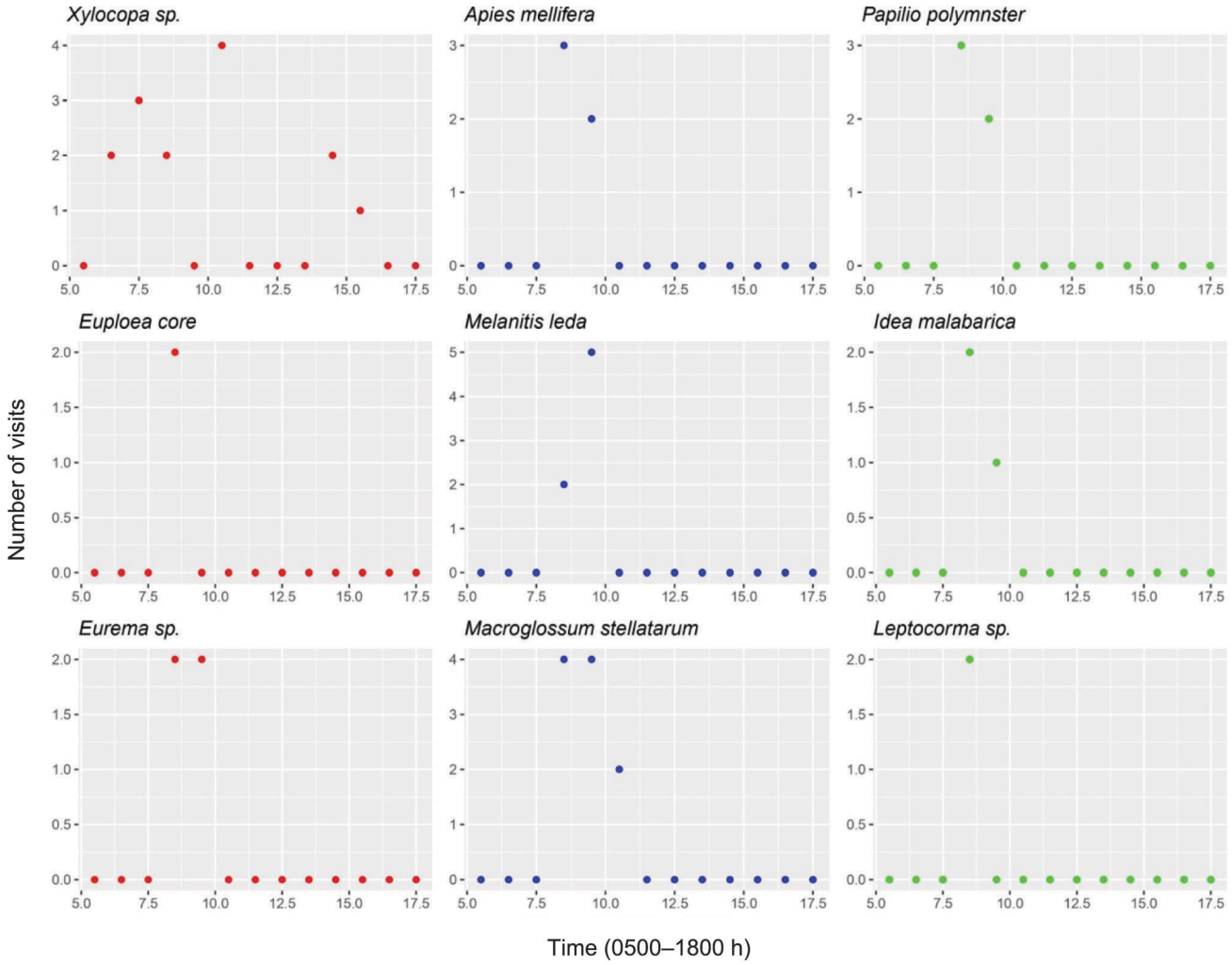

Figure 3. Flower visitors of *A. indica* from 0500 to 1800 h.

91.3%, 88% and 87.5% after 1, 2, 3 and 4 h respectively. A drastic decline to 20% was noted after 12 h. Hydrogen peroxide application followed by effervescence confirmed stigma receptivity up to 14 h, which later turned brown–black in colour, lost its turgidity and became non-receptive.

Pollen germination

At the time of anthesis, 58% of the pollen grains were found germinated in a 10% sucrose solution. A gradual decrease in pollen germination was observed at 47.8%, 23.2% and 9.74% after 1, 2 and 8 h respectively.

Pollen–ovule ratio

A flower contains 12–13 anthers and approximately 288 ± 51.3 pollen grains per anther. Hence pollen count per flower was estimated as 3744 ± 667. A flower has two ovules and hence the pollen–ovule ratio was estimated as 1872 : 1.

Pollination and insect interaction

The bagging experiment had failed; all the bagged inflorescences had fallen off. Pollinator documentation was done in 2017 and 2019. Observations were made from 0500 to 1800 h continuously. The peak time of pollinators incidence was recorded between 0800 and 1000 h and *Xylocopa* sp. was first visited during 0600–0700 h. *Apies mellifera*, *Idea malabarica*, *Eurema* sp., *Papilio polymnster*, *Euploea core*, *Graphium* sp., were the key pollinators seen multiple times (Figures 2 and 3). No flower visitors were recorded between 1100 and 1500 h. *Xylocopa* sp. was found foraging from 1500 to 1700 h. Though the *Macroglosum stelletarum* was not recorded during 2017, it was a frequent floral visitor in 2019.

Xylocopa sp., *M. stelletarum* and *A. mellifera* were found visiting many flowers, spending 2–3 sec per flower. Butterflies visited 2–3 flowers each time. Mouth parts of pollinators collected from the study site were observed through a compound microscope and photographs taken with a scanning electron microscope (SEM). The images

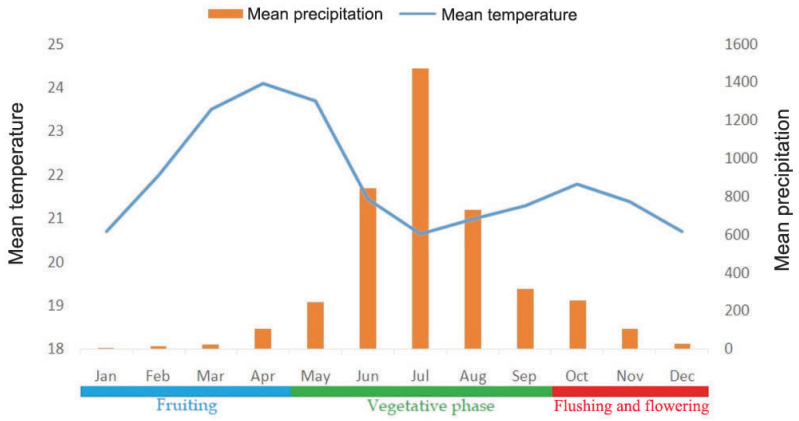

Figure 4. Phenological changes and climatic conditions of *A. indica* at the Kakkayam dam site, Kerala.

were compared with the SEM images of *A. indica* pollen grains, thus confirming their role as a pollinator (Figure 2 *d–j*). A troop of monkeys visited during the flowering season, jumping on the branches and causing flowers to fall. Inflorescence fall was recorded and the fallen inflorescence found with larvae (unidentified).

Cut-open fruits were found with Pyralidae larvae, which caused 30–40% fruit loss (Figure 2). The adult possibly lays eggs in the late flowering stage.

Discussion and conclusion

The major climatic variables that cause phenological changes are rainfall, temperature, insolation and water stress[18–21]. The flushing along with flowering in October–December in *A. indica*, reveals that flowering occurs after the monsoon season (Figure 4), (southwest monsoon, June–September in the southern Western Ghats). Kerala experiences a cool climate during the early weeks of October without any rainfall. Then the northeast monsoon sets in with lightning and thunder in the evening hours. Senescence of ripened fruits is observed during April and May. The hard seed coat delays seed germination *in situ*. However, at the start of the southwest monsoon, the seeds begin to germinate. The pollen–ovule ratio of the species supports cross-pollination (according to Cruden[17], the pollen–ovule ratio = 31.9–396 for autogamy).

The biotic interactions are influenced by phenology, population density and inter-population distance (for pollinators)[22]. Phenological variations mediate available pollinators, seed dispersal agents and florivorous insects[23,24]. This could also influence adaptation in primary and secondary consumers[25]. The most frequent pollinators are *Xylocopa* sp. and *A. mellifera.* Pollinator abundance and behaviour is the key factor in the seed set for entomophily-depending flowers[26]. Abscission of fruiting primordia (monkey-induced) was also observed. Honey bees are common pollinators found in target species, even though they are reported as less efficient because they collect pollen from various resources, resulting in deposition of pollen from multiple species on the stigma surface[27]. *A. indica* possesses coloured petals, nectar, scent and discoid-shaped stigma, which are found favourable for biotic pollination[3], it emphasizes cross-pollination of the flowers. The common pollinators of the target species are carpenter bees (*Xylocopa* sp.) and *A. mellifera*. *Xylocopa* sp. prefers medium-sized, yellow–white-coloured flowers with odour and nectar[28], which is characteristic of *A. indica* flowers. Carpenter bees are effective pollinators which support and maintain genetic variability as they travel long distances[28]. *A. mellifera* showed high efficiency in pollination[29], as reported in *Jatropha mollissima* and *Jatropha mutabilis*[30]. The large number of floral visitors reported in *A. indica* may be due to the exposed nature of the reproductive organs, as preferred by *A. mellifera.* Heavy loss in the developing inflorescence by the caterpillar lead to reduced number of flowers (adult to be identified).

The reasons for the reduced fruit set are stressful environment as well as resource limitation[31,32]. Apart from the minimal fruit set, the species showed restrictions in forming viable seedlings. The Pyralid larvae incidence in *A. indica* caused 50–70% loss in fruit set. Pyralid moths (snout moths) were also reported damaging cereals, dry fruits, etc.[33]. The fruits of *A. indica* are hard and therefore not eaten by birds or mammals. Generally, seeds of 70% of tropical forest species are dispersed by animals[34], but *A. indica* does not have any seed dispersers (the fruits are found only under the flowering tree).

The major limitation of this study is the extremely low number of flowering individuals, i.e. only two. One tree fell in 2017, and thus data collected from a single tree are presented here. The flowering phenology based on a single flowering individual may not be adequate to prove the behaviour of the species. However, it emphasizes the need for immediate conservation measures. Based on the microhabitat conditions, we conclude that less availability of sunlight is the reason for the low number of flowering

individuals. The flowered individuals are in forest edges facing the eastern side, while the non-flowering individuals are under a canopy of other species. In-depth studies on light and flowering relations are recommended.

1. Ramasubbu, R. and Felix, I. D., Reproductive biology of *Elaeocarpus blascoi* Weibel, an endemic and endangered tree species of Palni Hills, Western Ghats, India. *Curr. Sci.*, 2016, **110**(2), 234–240.
2. Kasi, K. S. and Ramasubbu, R., Phenological patterns, fruit predation, and seed dispersal in two endangered trees (*Elaeocarpus* spp.) of southern Western Ghats, India. *J. Asia-Pac. Biodivers.*, 2021, **14**, 275–282.
3. Dompreh, D., Michael, D. S. and Christopher, C. W., Reproductive biology of the critically endangered tropical tree *Talbotiella gentii*, *South. For.: J. For. Sci.*, 2015, **77**(2), 153–163; doi:10.2989/20702620.2014.1001674.
4. Evans, M. E. K., Eric, S. M. and Doria, R. G., Reproductive biology of three sympatric endangered plants endemic to Florida scrub. *Biol. Conserv.*, 2003, **111**, 235–246.
5. Stearns, S. C., Life history tactics: a review of the ideas. *Q. Rev. Biol.*, 1976, **51**, 3–47.
6. Roff, D. A., *The Evolution of Life Histories: Theory and Analysis*, Chapman & Hall, New York, USA, 1992.
7. Sreekala, A. K., Importance of plant reproductive biology in conservation. In National Conference on Bioresources: Conservation, Utilization and Future Prospects, The Gandhigram Rural Institute-Deemed University, Gandhigram, 2017.
8. IUCN, *Atuna indica*. In *The IUCN Red List of Threatened Species* 1998; https://dx.doi.org/10.2305/IUCN.UK.1998.RLTS.T38754A-10147928.en.
9. Asish, G. R., Deepak, M., Satheesh, G. and Indira, B., Phytochemical profiling and antioxidant activity of *Atuna indica* (Bedd.) Kosterm – an unexplored tree species reported from Western Ghats, India. *Int. J. Pharmacogn. Phytochem. Res*., 2013, 5.
10. Swarupanandan, K., Indira, E. P., Muralidharan, E. M., Pandalai, R. C. and Jose, P. A., Species recovery of *Dipterocarpus bourdillonii* and *Humboldtia bourdillonii*, two critically endangered endemic trees of Western Ghats, KFRI Research Report No. 463, Kerala Forest Research Institute, Kerala 2013, p. 86.
11. Sreekala, A. K., Pandurangan, A. G., Ramasubbu, R. and Kulloli, S. K., Reproductive biology of *Impatiens coelotropis* Fischer, a critically endangered balsam from the southern Western Ghats. *Curr. Sci*., 2008, **95**, 386–388.
12. Jose, P. A. and Pandurangan, A. G., Reproductive biology of *Ochreinauclea missionis* (Wall. Ex G. Don) Ridsd. an endemic and vulnerable tree from the Western Ghats, India. *Ann. For.*, 2012, **20**(2), 161–167.
13. Jose, P. A. and Pandurangan, A. G., Reproductive biology of *Gluta travancorica* Bedd. an endemic and threatened tree from the southern Western Ghats, India. *Ann. For.*, 2013, **21**(2), 165–171.
14. Gopalakrishnan, K. K. and Thomas, T. D., Reproductive biology of *Pittosporum dasycaulon* Miq. (family Pittosporaceae), a rare medicinal tree endemic to Western Ghats. *Bot. Stud.*, 2014, **55**, 15.
15. Dafni, A., Kevan, P. G. and Husband, B. C., *Practical Pollination Biology*, Enviroquest Ltd, Cambridge, Ontario, Canada, 2005.
16. Shivanna, K. R. and Rangaswamy, N. S., *Pollen Biology – A Laboratory Manual*, Narosa Publishing House, New Delhi, 1992.
17. Cruden, R. W., Pollen–ovule ratios: a conservative indicator of breeding system in flowering plants. *Evolution*, 1977, **31**, 32–46.
18. Dutta, G. and Ashalata Devi, Phenology and population structure of six tree species in tropical forest of Assam, northeast India. *Trop. Ecol.*, 2015, **56**, 343–399.
19. Ashton, P. S., Givinish, T. G. and Appanah, S., Staggered flowering in the Dipterocarpaceae: new insights into the floral induction and evolution of mast fruiting in seasonal tropics. *Am. Nat*., 1988, **132**, 44–66.
20. Stevenson, P. R., Phenological patterns of woody vegetation at Tinigua Park, Colombia: methodological comparisons with emphasis on fruit productions. *Caladonia*, 2004, **26**, 123–150.
21. Borchert, R., Soil and stem water storage determine phenology and distribution of tropical dry forest trees. *Ecology*, 1994, **75**, 1437–1449.
22. Lennartsson, T., Extinction threshold and disrupted plant pollinator interactions in fragmented plant populations. *Ecology*, 2002, **83**, 3060–3072.
23. Lobo, J. A., Quesada, M., Stonner, K. M., Fuchs, E. J., Herreri'As-Diego, Y., Rojas, J. and Saborio, J., Factors affecting phenological patterns of Bombacaceous trees in seasonal forests in Costa Rica and Mexico. *Am. J. Bot.*, 2003, **90**, 1054–1063.
24. McCall, A. C. and Irwin, R. E., Florivory: the intersection of pollination and herbivory. *Ecol. Lett.*, 2006, **9**, 1351–1365.
25. Van Schaik, C. P., Terborgh, J. W. and Wright, S. J., Phenology of tropical forests: adaptive significance and consequences for primary consumers. *Annu. Rev. Ecol. Syst.*, 1993, **24**, 353–377.
26. Larson, B. M. H. and Barret, S. C. H., A comparative analysis of pollen limitation on flowering plants. *Biol. J. Linn. Soc.*, 2000, **69**, 503–520.
27. Westerkamp, C., Honey bees are poor pollinators – why? *Plant Syst. Evol*., 1991, **177**, 71–75.
28. Raju, A. J. S. and Rao, S. P., Nesting habits, floral resources and foraging ecology of large carpenter bees (*Xylocopa latipes* and *Xylocopa pubescens*) in India. *Curr. Sci*., 2006, **90**, 1210.
29. Roubik, D. W., The value of bees to the coffee harvest. *Nature*, 2002, **417**(6890), 708.
30. Solomon Raju, A. J. and Ezradanam, V., Pollination ecology and fruiting behaviour in a monoecious species, *Jatropha curcas* L. (Euphorbiaceae). *Curr. Sci.*, 2002, **83**(11), 1395–1398.
31. Kollas, C., Vitasse, Y., Randin, C. F., Hoch, G. and Körner, C., Unrestricted quality of seeds in European broad-leaved tree species growing at the cold boundary of their distribution. *Ann. Bot.*, 2012, **109**, 473–480.
32. Friedman, J. and Barrett, S. C. H., The evolution of ovule number and flower size in wind-pollinated plants. *Am. Nat.*, 2011, **177**, 246–257.
33. https://en.wikipedia.org/wiki/Pyralidae (accessed on 2 February 2022).
34. Tadwalkar, M. D. *et al*., Dispersal modes of woody species from the northern Western Ghats, India. *Trop. Ecol.*, 2012, **53**(1), 53–67.

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