

Floral biology and reproductive effort of andromonoecious *Aesculus indica* Wall. ex Camb. Hook (Hippocastanaceae)

Priya Pradhan

Veer Chandra Singh Garhwali Uttarakhand University of Horticulture and Forestry

Arun Sukumaran

Veer Chandra Singh Garhwali Uttarakhand University of Horticulture and Forestry

Vinod Prasad Khanduri (✉ khandurivp@yahoo.com)

Uttarakhand University of Horticulture and Forestry <https://orcid.org/0000-0002-3858-7121>

Research

Keywords: Andromonoecy, staminate flowers, protogyny, Stigma Receptivity, breeding system, ambophily, reproductive success, crown layers

Posted Date: April 10th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-21597/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Background Andromonoecious is an unusual sex-expression in trees; in which an individual plant bears both functionally staminate and hermaphrodite flowers on the inflorescences. The significant effects of crown layers on reproductive success have not been studied so far. This study aims to investigate the effect of crown layers on floral biology and reproductive effort of *Aesculus indica*. Results The results revealed that the peak period of peak period of anthesis was between 06:00-08:00 h of the day. Male flower production was predominantly higher as compared to the perfect flower on the inflorescence. There was no significant variation between total pollen production in staminate and perfect flowers. The features like protogyny and inter-level asynchrony promote xenogamy, however, the intra-level asynchrony resulted geitonogamy. Controlled pollination treatment revealed the existence of self-incompatibility in flowers. Pollination syndromes of flowers are supporting ambophily. A trend of consistent improvement in reproductive success from lower canopy layers to upper canopy layers in trees was recorded. The crown layers have a significant impact on flower production, fruit, and seed set. Conclusions Increment in male flower production due to the increment in crown is a mechanism of reproductive assurance as a pollen donor and pollinator recipient and also due to the differential cost of expenditure of reproduction in crown layers.

Background

The genus *Aesculus* (family: Hippocastanaceae) comprises of thirteen species, numerous botanic varieties, cultivars and natural hybrids spread across the Northern hemisphere, mainly in temperate climates. The species are organized into 5 sections *Aesculus*, *Calothyrsus*, *Macrothyrsus*, *Parryana*, and *Pavia* (Hardin 1956). *Aesculus indica* Wall. ex Camb. Hook. (Indian horse chestnut) is a large, rounded tree and falls under the section *Calothyrsus*. *A. indica* is common along the Himalayan lowlands in the elevations of 1000-3000m, in the forests and shady valleys across Northern Afghanistan, Pakistan, Kashmir, North India and Nepal (Troup 1921). *A. indica* is the most climatically sensitive species of all *Aesculus*. It has the widest acceptable pH range of 6.1 to 7.5 (Sternberg and Wilson 1995). It is widely grown as a shade tree for parks, arboreta, college campuses and home landscapes (Dirr 1990) and also recommended for buffer strips. The bark of *A. indica* has been used as a tonic because of its astringent properties (Hooker 1859). The wood is used to make utensils and pots and is also used for making, packing-cases, tea boxes and decoration articles (Rajasekaran and Singh 2009).

The genus *Aesculus* has an unusual reproductive strategy including a large portion of functionally staminate flowers, and a high microspore to mature seed ratio. The flowering biology of the genus characterizes andromonoecious (having perfect and staminate flowers on the same plant). Hardin (1956) stated that functionally staminate flowers have a vestigial gynoecium with an undeveloped ovary and stunted style. Complete flowers have fully developed androecium and gynoecium. Pistillate inflorescences were found infrequently in *A. hippocastanum* and in *A. pavia*, where two plants out of many hundreds had only functionally female flowers (Bertin 1980). Proportions of female-fertile flowers are as low as 0.1% in *Aesculus* (Sedgley and Griffin 1989). The sex ratio of different *Aesculus* species was reported as 1.3 to 9.3% for *A. turbinata* (Suo et al. 1995); 1.1% for *A. pavia*, (Bertin 1980); <5% for *A. californica* (Benseler 1968); and 7.0% for *A. sylvatica* (Coker and Totten 1937). The empirical studies on the reproductive biology of *A. indica* are

required in order to understand the variability and relationships among floral morphology, mating system and floral visitors under different ecological contexts.

Yet, there is no study regarding the reproductive effort of tree species under various layers of the crown. Reproductive success may be varied among crown layers of the tree. The possible assumptions are the differential allocation of photosynthates in crown layers or influence of sunlight on fruit and seed set or variation in effective pollination and fertilization success in crown layers would be expected. Suo et al. (1995), using *A. turbinata*, reported that panicles from the upper crown (presumably receiving optimum photosynthetically active radiation) were longer and had a greater number of flowers per panicle and a higher sex ratio than those from lower portions of the crown. Furthermore, fruit development depends strongly on photosynthesis and light availability (Brady 1987; Raven et al. 1992; Taiz and Zeiger 1998). Leaves in the upper part of trees shade those below; conceiving a vertical stratification of light within the tree. Consequently, upper leaves are expected to produce more photosynthates than lower, shaded leaves.

We investigated the floral morphology and biology, pollination and breeding systems of *A. indica* for addressing the following questions: (1) Do any difference in reproductive success and reproductive potential in terms of fruit and seed set percentage in different crown layers? (2) Is there any significant difference between the production of staminate and bisexual flowers in the sample trees? (3) What is the functional role of staminate flower? (4) Whether pollen production in staminate flower and perfect flower are equal or not? (5) What is the main pollination system of study species? (6) What are the major floral visitors and how they take part in reproduction?

Methods

Study area

This study was conducted in the natural moist temperate forests of Ranichauri area situated adjacent to the College of Forestry, Ranichauri, Tehri Garhwal (latitude 30° 18' N, longitude 78° 24' E, altitude between 1750-2100 m asl) in the state of Uttarakhand, North West Himalaya, India. The nearby vegetation is comprises of the temperate broad leaved and coniferous forest, viz. *Myrica esculenta*, *Pyrus pashia*, *Plantanus orientalis*, *Quercus leucotrichophora*, *Rhododendron arboreum*, *Cedrus deodara* and *Pinus roxburghii*. The study was carried out from April 2016 to November 2016. The study area typically experiences a moist temperate type of climate with chilled winters. The mean monthly maximum and minimum temperature during the study period was oscillated between 7.0⁰C and 29.9⁰C. The trees of *A. indica* were selected under varying microclimatic conditions. The trees were chosen on the basis of accessibility, convenience, and free from biotic pressure.

Floral morphology and anthesis

Twenty inflorescences at flower bud stage were selected and marked on the sample trees for observation. Basic stages of flower development were defined by a detailed examination of the flowers. To examine anthesis, flowers were observed frequently at 2h interval within a day between 0600 and 1600 h. during the whole period of study and noted the number of flowers opened. Opened flowers were marked with a permanent marker to avoid error due to duplication and overcounting at every two hours intervals. To

elucidate the anther structure and morphological changes of the stigma, flowers were collected and observed under a microscope. Regular phenological observations were made for individual sample trees.

Pollen production

Pollen estimation was done separately for both male flower and perfect flower. The pollen production per flower in trees was assessed by using the noon loop method (Khanduri 2012). Anthers were obtained from closed flowers just prior to anthesis and were laid into a test tube containing 5 drops of 5% glycerol, crushed with a glass rod and pollen grains were suspended in the test tube. The number of pollen grains was counted under a compound microscope. Pollen production per anther was determined with the formula $P = (T_p/N) \times n$ where, T_p is the summation of the total number of counted pollen grains in all the five drops, N is the total number of samples (slides) used for counting, and n is the total number of drops. Production of pollen grains per flower was estimated by multiplying the number of pollen grains per anther with the number of anthers per flower. Mean pollen production of a tree was determined by using the equation $TP = \sum N \times F \times A \times P$ where TP is the total pollen grains per tree, $\sum N$ is number of inflorescence per tree, F is the average number of flowers per inflorescence, A is the average number of anthers per flower, P is the average number of pollen grains per anther.

Stigma receptivity and pollen ovule ratio

Stigma receptivity was determined by immersing stigmas of flowers in 3% aqua's hydrogen peroxide; bubbling indicating peroxide activity and stigma receptivity (Carrington et al. 2003). Flowers from different inflorescences opened at the almost same time were immersed at two hours intervals up to thirty-eight hours to determine the average duration of stigma receptivity. Pollen-ovule (P/O) ratios were determined by dividing the estimate of the number of pollen grains per flower by the number of ovules per flower (Cruden, 1977). To determine ovule number cross section of the ovary was taken and counted the ovule number.

Pollen viability

The viability of the pollen grains was tested under *in-vitro* by using the acetocarmine staining method (Alexander 1969). We used anthers from fresh flowers prior to the anthesis. Pollen grains were dusted onto a clean microscopic slide to which equal amount of acetocarmine (0.5%) and glycerol was added and warmed gently. The slides were incubated for 10-15 minutes. Stained pollen grains within the microscopic slide were counted as viable pollen while shrivelled, empty and weakly stained were recorded as non-viable.

Pollen germination

Pollen germination of *A. indica* was tested separately for freshly collected pollen grains from both male and perfect flowers under *in-vitro* condition in sucrose solutions (germination media) with the concentrations of 10%, 20%, and 30 %. Pollen grains were placed in Petri dishes containing germination media (20ml) and maintained at 20.4 °C (room temperature) for 48 hours. Germinated pollen grains were observed under the binocular microscope in each solution. The pollen grains were considered germinated when the pollen tube length was greater than the diameter of the pollen grain (Tuinstra and Wedel 2000). Germination rate was quantified as the percentage of germinated pollen grains per 100 evaluated grains.

Breeding system and floral visitors

A total of 100 flowers from each sample trees were subjected to four types of treatments (25 flowers for each treatment), 1) Open pollination–the flowers were left without any intervention (no bag, no artificial pollination) and observed up to fruit set, 2) obligate self-pollination – flower buds were bagged throughout the flowering period and observed up to fruit set, 3) Open cross-pollination- removing the anthers from the flowers prior to anthesis and was observed for fruit set, 4) Apomixes- removing both the anther and the stigma of the flowers in bud stage and observed for fruit set.

Pollinator observations were carried out throughout the day between 06:00- 16:00 hours on each experimental tree continuously on 8 days in the month of May. Insects visiting the inflorescences were observed for their foraging behaviour, the number of individuals and duration of the visit. The insects were captured using insect trapping net (Sweep net) and polythene bags, every hour during the foraging period of flower visitors (06:00h to 18:00h). Unidentified insects' specimens were dried and placed into separate rectangular papers used for further identification. The double mounting method has opted for small insects.

Reproductive Success in different crown layers

The crown length of the selected sample trees was measured using Haga altimeter. The total crown length was divided into three equal proportions named upper canopy layer (UC), middle canopy layer (MC), and lower canopy layer (LC) (Fig. 1). The total number of inflorescences in each canopy layer was counted manually and was multiplied by the average number of flowers per inflorescence to get the total number of flowers. Fruit set and seed set were calculated separately for each canopy layer. Here fruit set is defined as the proportion of flowers that developed into fruits in each inflorescence. Seed set is defined as the proportion of ovules that developed into seeds in all the mature fruits within an inflorescence. Twenty inflorescences each in upper, middle and lower canopy were tagged in each sample tree and observed for the fruit formation. The average fruit set in each canopy layer was calculated by multiplying the number of fruit set per inflorescence by the total number of inflorescence in each canopy layer and expressed in percentage. Similarly, seed set per tree was calculated as, the average number of seed set per inflorescence multiplied by the total number of inflorescence within the canopy. The female reproductive success of tree was calculated by multiplying the mean number of fruits per plant by the mean number of seeds per fruit (Dafni 1992).

Data analysis

Mean \pm standard deviations of the mean were calculated for all the measurements. The effect of time and tree to tree variation on flower opening was examined by two-factor analysis of variance (ANOVA) with time and tree to tree difference as fixed effects. Student's t-test (test for difference in mean) was performed for assessing the difference in pollen production in staminate and perfect flowers, number of staminate and perfect flowers within the inflorescence. The effect of time and sucrose concentration on pollen germination was also examined with two-way analysis of variance (ANOVA) with time and concentration of the solution as fixed effects. Among crown layers variation in the production of inflorescence, number of flowers, number of fruit per inflorescence, and reproductive success was assessed by single-factor ANOVA with crown layers as fixed effects. The effect of pollination treatments on fruit set was also verified by single-factor ANOVA with treatment as fixed effects.

Results

Floral morphology and anthesis

The sex expression in *A. indica* is andromonoecious in which the inflorescences bear both staminate and perfect flowers. There was a marked difference in the number of staminate flowers and perfect flowers within the inflorescence ($t = 10.60$, $P \leq 0.05$, d.f.=18). Majority of the flowers are staminate, with the pistil absent or rudimentary. The inflorescence is compound raceme (Tyrsus) bearing terminal panicles, broad at the base forming a pyramidal shape. The flowers are pedicellate, zygomorphic, the number varied from 341 ± 65 to 441 ± 74 per inflorescence, the number of perfect flowers per inflorescence is 13 ± 3 to 20 ± 6 . Flower composed of the polypetalous corolla and has four petals clawed which are narrow at the base and broad at the top (unequal in breadth) the place of 5th usually vacant. The sepals are fused (gamosepalous), tubular, tomentose and have five lobes. The androecium includes seven stamens (occasionally 6, 8 and 9) which are filiform, tomentose, narrow and oblong. It is curved upward and longer than the petals. The gynoecium is hypogynous, simple and slender and sessile ovary having 6 ovules arranged in three cells with two ovules each.

Reproductive phenological observations showed that the flower bud initiation takes place in the month of March and the fruit set appeared in the last week of April and fully ripened during October to November thus took 7.5 months for the formation of zygote. Inflorescence was commenced in March and took 27 ± 2 d for enlargement of buds, individual bud took 8-12 d for complete development (Fig. 2), the mean time taken for the complete blooming of one inflorescence was 56 ± 2 d, and mean number of days for fruit development from bud initiation was 142 ± 5 d. Flowers are opened in acropetal pattern to be noted from the second week of April to till June. The peak period of anthesis was recorded between morning hours 06:00 to 08:00 h of the day (Table 1). The effect of tree to tree difference on anthesis was not significant (Table 2, $F=3.26$, $P \leq 0.05$), however, there was a significant effect of time on anthesis (Table 2, $F=968.55$, $P \leq 0.05$). Anther dehiscence begins after 5-6 h of the anthesis.

Pollen production

The total flower production in the sample trees was oscillated between 18804 ± 3611 to 151906 ± 24179 . Pollen grains per stamen (7597 ± 91 to 7739 ± 169 for male flowers and 7590 ± 56 to 7781 ± 170 for perfect flowers). Pollen production between male and perfect flowers did not differ significantly ($t = 0.261$, $P = 0.05$). The mean pollen production per flower, inflorescence and tree was ranged between 53183 ± 643 to 54177 ± 1189 , 18363790 ± 476673 to 23914032 ± 524987 and 1010008499 ± 26217045 to 8078958841 ± 97679046 , respectively.

Stigma receptivity and pollen ovule ratio

The inflorescence of *A. indica* was protogynous female receptivity lasts about 5-6 d and shows distinct morphological changes. The stigma looked fresh and pink in colour throughout its receptive phase. Eventually, stigma becomes dry, shrink and brown in colour which made clear the cease of receptivity.

There are six ovules per flower; hence the P/O ratio varied from 8855 ± 65 to 9078 ± 198 pollen grains per flower for perfect flower and 8863 ± 107 to 9029 ± 198 pollen grains per flower for the male. In addition, according to Cruden (1977) classification, the P/O ratio of *A. indica* falls within the reported range of Xenogamy.

Pollen viability and germination

The percentage of viable pollen per slide was ranged between $87.54 \pm 4 \%$ to $90 \pm 0.8 \%$ for perfect and $85.3 \pm 3 \%$ to $89.6 \pm 4 \%$ for male flowers. The effect of time and concentration of solution on pollen germination was found to be significant for perfect flowers ($F=48.73$, $F=18.10$) and male flowers ($P \leq 0.05$, $F=67.84$, $F=31.13$, $P \leq 0.05$) (Table 2). The maximum germination of 88.17% for perfect flowers and 88.21 % for male flowers were observed in 20% sucrose concentration, followed by (83.33% for perfect flowers and 84.19% for male flowers) in 10% sucrose.

Breeding systems and floral visitors

There was a significant effect of treatment on fruit set (Table 2, $F=13.833$, $P=0.00156$). A marginal difference on fruit set was observed between the open-pollination and the open cross-pollination. The flowers were failed to set fruit under the treatment of obligate-self pollination and apomixes, indicating the need for pollen transmission vectors.

A diverse array of insects belongs to the orders Hymenoptera, Thysanoptera and Lepidoptera were observed visiting the flowers of *A. indica* during the 8 d observation period (Table 3). Most visiting were observed in morning hours between 08:00-10:00 h. The most frequent visitors in flowers were *Bombus spp.* and Thrips. There was little difference in the visitation rates of the insects to staminate and perfect flowers. The inflorescence received most *Bombus spp.* in the morning (06:00- 08:00 h) and rarely in afternoon. Bumblebees tended to visit the outward facing flowers and the flowers at the base of the panicle first. *Tagiades menaka* was found more frequently in the evening (16:00 - 18:00 h). The butterflies were observed feeding at the top of the inflorescence and visiting both buds ready to open and open flowers, could be considered as a marginal pollinator. Hummingbird moth was also observed visiting the flowers; they tended to visit the panicle apex first. In aggregate, the activity of honey bees, butterflies, and bumblebees have coincided with the peak period of the flower opening, which strongly supporting the role of insect in pollination.

Reproductive success in different crown layer

The production of inflorescence and production of flowers did not differ significantly on crown layers (Table 2, $F= 1.323$, $P=0.33341$, $F=1.272$, $P=0.3462$), fruit set within inflorescence showed highly significant difference between crown layers (Table 2, $F=12.24$, $P=0.0076$) in trees. We also have noticed the trend of increasing the number of inflorescence and flowers production from the lower canopy (LC) to upper canopy layer (UP) and consequently enhanced the fruit set in middle and upper canopy layers (Table 4). Surprisingly, there was a significant difference in reproductive success (proportion of the number of flowers converted into fruit) between crown layers in trees (Table 2, $F=14.237$, $P=0.0052$). Although there are 6 ovules in each ovary, successful pollination invariably leads to the formation of only one seed in a fruit. Only the ovule located at

either the first or the second position towards the stylar end matures into a seed. The remaining ovules abort results very low reproductive potential in trees (0.167).

Discussion

This paper reports the first comprehensive study of floral biology and reproductive effort of *A. indica*. Many of the workers have investigated the various reproductive aspect of the genus *Aesculus* including the species *A. indica*, whilst many parts being still unknown. The andromonoecious sex-expression in *A. indica* is depicting significant difference in the number of male flowers and perfect flowers in an inflorescence. The surplus production of male flower maximises pollinator attraction and function as a pollen donor to fertilise another hermaphrodite flower which enhance the female and male fitness within the population. The study conducted by past workers stated that staminate flowers may have two selective advantages. First, they provide a pollen surplus that enhances male fitness (Bertin, 1982; Anderson and Symon 1989; Podolsky 1993; Connolly and Anderson 2003). Secondly, staminate flowers increase floral display that improves pollinator attendance, which in turn benefits female fitness (Bertin 1982; Anderson and Symon 1989; Spalik 1991). Staminate flowers and perfect flowers were morphologically alike except incomplete development of pistil in staminate flowers, the more advanced study is required to understand the floral sexual dimorphism in this species.

In *A. indica* diurnal pattern of anthesis has been witnessed; the peak period of anthesis coincides with the maximum activity of the floral visitors. Clearly, flowering time of each species is genetically fixed, whilst, which is highly variable with the environmental factors, mainly precipitation, temperature and relative humidity (Hegazy et al. 2017; Amano et al. 2010; Khanduri et al. 2008). In addition, the flowering pattern of *A. indica* was found to be asynchronous *i.e.* buds and flowers are at different stages of development even on the same tree. Similar results were also reported by Bangarwa (1993) in *Dalbergia sissoo*, Khosla et al. (1982) in *Bombax ceiba*. Individuals with asynchronous flowering decrease reproductive output, the amount of pollen, the number of pollen donors and the levels of outcrossing compared to individuals blooming during the same period (Schwartz, 2003).

There was no significant variation in pollen production between perfect and staminate flowers. So it can be inferred that there would be differential cost of expenditure for production of staminate and perfect flowers, probably the production of flowers serves only pollen source would be less expensive. Therefore, the trees inevitably produce more staminate flowers to increase the pollen output and female fitness. Similar findings were also reported by Reale et al. (2006). The hermaphrodite (perfect) flowers possess protogynous dichogamy (female phase preceding the male phase). Stigmas were receptive prior to the anthesis and anther dehisced 5–6 h after the anthesis indicating that the hermaphrodite flowers were well adapted to outbreeding and declined the chances of auto-pollination in *A. indica*. Protogynous dichogamy is one of the mechanisms promoting outcrossing in *Acacia* (Sedgley 1987). At anthesis, anthers were not dehisced results greater chances of viable pollen from outside may deposit over the stigma and get fertilized. The features like marked protogyny and inter-level asynchrony promotes xenogamy, however, the intra-level asynchrony perhaps results in gietonogamy.

A high percentage of pollen viability from both hemaphrodite and staminate flowers exert a direct control on pollen-stigma interactions (Heslop-Harrison et al. 1984; Heslop-Harrison and Heslop-Harrison 1992), fruit set

and gene flow (Dafni and Firmage 2000). Pollen germination was significant with time and concentration of germination media. The pollen germination was increasing with increase in time till 5 h, later there was a steady decline in the germination rate. 20% sucrose concentration exhibited maximum pollen germination, as sucrose is the carbohydrate source for pollen germination and tube growth (Tupy 1960). The results on controlled pollination reveal predominantly self-incompatibility in *A. indica* as no fruit set was observed from autogamous (self-pollination) pollination. Hence pollination is completely dependent on vectors for its reproductive assurance. Interestingly, fruit set under open pollination was almost 10% less than that of open-cross pollination which would be due to the absence of out-crossed pollen. Kalinganire et al. (1996) mentioned that the poor fruit set following natural open-pollination is mainly due to the absence of outcrossed pollen. This is probably due to low visitation rates by effective pollinators. Since there was no spontaneous autogamy, as indicated by lack of fruit set in completely bagged flowers.

A. indica was found to possess all the characters of anemophily syndrome such as long-exserted terminal inflorescence, small nectar less flowers, high P/O ratio, small and smooth pollen produced in large numbers (Stanley and Kirby, 1973; Faegri and van der Pijl 1979; Sharma and Khanduri 2007). The existence of protogyny, asynchronous flowering and self-incompatibility rules out the possibilities of self- (intrafloral or geitonogamous) pollination in the canopies. The present studies on floral visitors have revealed that the flowers of *A. indica* were frequently being visited by five insect species from three orders such as Hymenoptera, Thysanoptera, and Lepidoptera. It can be inferred that the pollination system in *A. indica* had adapted to support both entomophily and anemophily, the occurrence of which is referred to as ambophily (Yamasaki and Sakai 2013).

Most of the foragers was visiting the flowers during the peak period of blooming (08:00–10:00 h) of the day. It was apparent that anther dehiscence occurred 5–6 h later the anthesis and not coinciding with the pollinator activity, clearly indicating that the foragers visiting the flower inadvertently. Moreover, there was no difference in visitation rate of insects on both staminate and perfect flowers. The higher number of male flowers may reduce the probability of bisexual flower visits consequently reduces the fruit set. Alternatively, pollen grains of 1 day old opened flowers were contributing as pollen donor to freshly opened flowers, augmenting maximum cross-fertilization. Despite, the frequent incidents of Bees, Thrips and Butterflies, flowers were assumed to increase the chances of pollen theft and negatively affect the seed production (Bertin 1980; Arizmendi et al. 1996; Irwin et al. 2001; Lara and Ornelas 2001). Because of these reasons insects cannot be considered as an effective pollinator in *A. indica*. Furthermore, frequent visit of more number of insects to the flowers increase the pollen concentration in the air, which assisting wind pollination in *A. indica*. The assistance of insects in anemophily was recorded in *Phyllostachys nidulari* (Huang et al. 2013); *Plantago lanceolata* (Clifford 1962); and *Mallotus* (Yamasaki and Sakai 2013). Therefore, the pollination syndrome of *A. indica* supports both wind (anemophily) and insect (entomophily) pollination.

Unlikely, we found that there was a significant difference in the proportion of flowers set into fruit (reproductive success) in canopy layers. Reproductive success was found to be markedly high in upper canopy layers as compared to lower layers. Exactly what causes the variability in fruit production in crown layers? Clearly, a number of factors affecting the fruit production, (1) differential cost of expenditure of reproduction in crown layers results inadequate translocation of resources (2) ample amount of light availability and temperature increase the photosynthetic rate and reproductive success (3) pollen limitation

arises from the preferential activity of pollinator (4) natural selection pressure. Similar findings were reported by a number of workers (e.g. Fruit development depends strongly on photosynthesis and light availability (Brady 1987; Raven et al. 1992; Taiz and Zeiger 1998); competition by the surrounding neighborhood of plants for those resources (Lee and Bazzaz 1982; Graham et al. 2003); zones of the canopy that receive more sunlight are expected to distribute more photosynthates locally, from the leaf to the adjacent fruit: local translocation of resources hypothesis (Lynch and Gonzalez 1993; Proietti et al. 2000; Raven et al. 1992; Taiz and Zeiger 1998).

In *A. indica*, the fruit set and seed set rates are the same due to the production of 1-seeded fruits (Drupe). It is evident that fruit and seed production is very low when compared to the numbers of flowers and ovules. Lee (1988) and Guitián et al. (1996) proposed several factors that may limit fruit production, including extrinsic causes (pollen limitation, herbivory, frost, etc) and intrinsic causes (genotypes, stored resource content, etc). The most common factor reported in the literature is resource limitation (e.g. Stephenson 1981; Berjano et al., 2006). Furthermore, we speculated that the low rate of reproductive potential (S/O) was due to the existence of a high degree of ovule abortion. High rates of ovule abortion in multi-ovulated species are still conjectural. It could be argued that the self-incompatibility and intra-ovule competition within the ovary would likely to be the primary reason for ovule abortion under pollen surplus condition in *A. indica*.

Conclusions

This study indicates that *A. indica* is self-incompatible and outcrossing species. Surplus male flower production is likely an ovary reserve mechanism as a pollen donor and pollinator attractant. Ambophily (anemophily as well as entomophily) is the primary pollination mechanism in *A. indica*. We found that crown layers have a significant impact on flower production, fruit, and seed set presumably due to the differential cost of expenditure of reproduction in crown layers. Floral dimorphism, ovule-abortion, and productivity of crown layers are the subject of subsequent study in *A. indica*.

Declarations

Acknowledgements

The authors are thankful to C. Vanlalruata, P.V. Chand, R. Bhandari, and K. Dabral for their consecutive assistance in field works.

Author's contributions

PP, AS and VPK conceived the study; PP and AS set up the experiment, conducted field work and drafted the initial manuscript; VPK revised the drafts. All authors read and approved the manuscript.

Funding

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

No existing ethics and consent of interests.

Consent for publication

NA

Competing interests

The authors declare that they have no competing interests.

References

- Amano T, Smithers RJ, Sparks TH, Sutherland WJ (2010) A 250-year index of first flowering dates and its response to temperature changes. *Proceedings of the Royal Society B: Biological Sciences* 277:2451-2457.
- Alexander MP (1969) Differential staining of aborted and non-aborted pollen. *Stain Technology* 44: 117.
- Anderson GJ, Symon DE (1989) Functional dioecy and andromonoecy in *Solanum*. *Evolution* 43: 204–219.
- Arizmendi MDC, Dominguez CA, Dirzo R (1996) The role of an avian nectar robber and of hummingbird pollinators in the reproduction of two plant species. *Functional Ecology* 10: 119-127.
- Bangarwa KS (1993) Studies on provenance variation, progeny testing and reproductive biology in *Dalbergia sissoo* Roxb. Ph.D. Thesis, CCS HAU, Hisar, Haryana, India.
- Benseler RW (1968) Studies in the reproductive biology of *Aesculus californica*. Ph.D. Dissertation. University of California, Berkeley, CA.
- Berjano R, De Vega C, Arista M, Ortiz PL, Talavera S (2006) A multi-year study of factors affecting fruit production in *Aristolochia paucinervis* (Aristolochiaceae). *American Journal of Botany* 93: 599–606.
- Bertin RI (1982) The evolution and maintenance of andromonoecy. *Evolutionary Theory* 6: 25–32.
- Bertin RI (1980) The reproductive biologies of some hummingbird-pollinated plants. Ph.D. Dissertation. University of Illinois, Urbana.
- Brady CJ (1987) Fruit ripening. *Annual Review of Plant Physiology* 38: 155–178.
- Carrington ME, Gottfried TD, Mullahey JJ (2003) Pollination biology of saw palmetto (*Serenoa repens*) in Southwestern Florida. *Palms* 47: 95-103.
- Clifford HT (1962) Insect pollination of *Plantago lanceolata* L. *Nature* 193, 196.

- Coker W, Totten H (1937) Trees of the southeastern states, 2nd addition. Chapel Hill: The University of North Carolina Press.
- Connolly BA, Anderson GJ (2003) Functional significance of the androecium in staminate and hermaphroditic flowers of *Solanum carolinense* (Solanaceae). *Plant Systematics and Evolution* 240: 235–243.
- Cruden RW (1977) Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. *Evolution* 31: 32–46.
- Dafni A (1992) Pollination ecology. A practical approach. Oxford University Press, New York, New York, USA.
- Dafni A, Firmage D (2000) Pollen viability and longevity: practical, ecological and evolutionary implications. *Plant System Evolution* 222: 113–132.
- Dirr M (1990) Manual of woody landscape plants: their identification, ornamental characteristics, culture, propagation and uses. 4th ed. Stipes Publishing Co., Champaign, IL.
- Faegri K, van der Pijl L (1979) The Principles of Pollination Ecology. Pergamon Press Ltd., Oxford, England
- Graham EA, Mulkey SS, Kitajima K, Phillips NG, Wright SJ (2003). Cloud cover limits net CO₂ uptake and growth of a rainforest tree during tropical rainy seasons. *Proceedings of the National Academy of Sciences*. 100: 572-576.
- Guiti  n P, Guiti  n J, Navarro L (1996) Fruit set, fruit reduction and fruiting strategy in *Cornus sanguinea* (Cornaceae). *American Journal of Botany* 83: 744–748.
- Hardin JW (1956) Studies in the Hippocastanaceae II. inflorescence structure and distribution of perfect flowers. *American Journal of Botany*. 43: 418-424.
- Hegazy AK, Kabi  l HF, Al-Rowaily SL, Lovett-Doust L, Al Borki AES (2017) Plant communities and reproductive phenology in mountainous regions of northern Libya. *Journal of Forestry Research* 28:741–761.
- Heslop-Harrison J, Heslop-Harrison Y (1992) Intracellular motility, the actin cytoskeleton and germinability in the pollen of wheat (*Triticum aestivum* L.). *Sexual Plant Reproduction* 5: 247–255.
- Heslop-Harrison J, Heslop-Harrison Y, Shivanna KR (1984) The evaluation of pollen quality and a further appraisal of the fluorochromatic (FCR) test procedure. *Theoretical and Applied Genetics* 67: 367–375.
- Hooker WJ (1859) Curtis botanical magazine: Plants of the Royal Gardens of Kew: Vol. 85. Lovell Reeve, London
- Huang SQ, Xiong YZ, Barrett SC (2013) Experimental evidence of insect pollination in Juncaceae, a primarily wind-pollinated family. *International Journal of Plant Science* 174: 1219–1228.

- Irwin RE, Bronstein JL, Manson JS, Richardson L (2001) Nectar robbing: ecological and evolutionary perspectives. *Annual Review of Ecology, Evolution and Systematics* 41: 271-292.
- Kalinganire A, Harwood CE, Simons AJ, Moncur MW, Slee M (1996) Reproductive ecology of *Grevillea robusta* in western Kenya. In: Dieters MJ, Matheson AC, Nikles DG, Harwood CE, Walker SM, eds. *Tree improvement for sustainable forestry. Proceedings of the QFRI-IUFRO Conference, Caloundra, Queensland, Australia. 27 October-1 November 1996*, 238-243.
- Khosla PK, Shamet GS, Sehgal RN (1982) Phenology and breeding system of *Bombax ceiba* Linn. In: *Improvement of Forest Biomass*, (P.K. Khosla, eds.), ISTS, Solan, 41-49.
- Khanduri VP (2012) Annual variation in floral phenology and pollen production in a 25-year-old plantation of *Tectona grandis*. *Nordic Journal of Botany* 30: 82–89
- Khanduri VP, Sharma CM, Singh SP (2008) The effects of climate change on plant phenology. *The Environmentalist* 28:143-147
- Lara C, Ornelas JF (2001) Preferential nectar robbing of flowers with long corollas: experimental studies of two hummingbird species visiting three plant species. *Acta Oecologica* 128: 263-273.
- Lee TH (1988) Patterns of fruit and seed production. In: Lovett Doust J, Lovett Doust L, eds. *Plant reproductive ecology*. Oxford, UK: Oxford University Press, 179–202.
- Lee TD, Bazzaz FA (1982) Regulation of fruit and seed production in an annual legume, *Cassia fasciculata*. *Ecology* 63: 1363-1373.
- Lynch J, Gonzalez A (1993) Canopy nutrient allocation in relation to incident light in the tropical fruit tree *Borojoa patinoi* (Cuatr.). *Journal of American Society of Horticultural Science* 118: 777–785.
- Podolsky RD (1993) Evolution of a flower dimorphism: how effective is pollen dispersal by 'male' flowers? *Ecology* 74: 2255–2260.
- Proietti P, Palliotti A, Famiani F, Antognozzi E, Ferranti F, Andreutti R (2000) Influence of leaf position, fruit and light availability on photosynthesis of two chestnut genotypes. *Scientia Horticulturae* 85: 63–73.
- Rajasekaran A, Singh J (2009) Ethnobotany of Indian horse chestnut (*Aesculus indica*) in Mandi district, Himachal Pradesh. *Indian Journal of Traditional Knowledge* 8: 285-286.
- Raven PH, Evert RF, Eichhorn SE (1992) *Biology of plants* (5th ed.). New York: Worth Publishers
- Reale L, Sgromo C, Bonofiglio T, Orlandi F, Fornaciari M, Ferranti F, Romano B (2006) Reproductive biology of Olive (*Olea europaea* L.) DOP Umbria cultivars. *Sexual Plant Reproduction* 19: 151- 161.
- Schwartz MD (2003) *Phenology: An Integrative Environmental Science*. Springer Dordrecht Heidelberg New York, London.

Sedgley M (1987) Reproductive biology of Acacia. In: Turnbull JW, ed. Australian Acacia species in developing country. Canberra: Australian Centre for International Agricultural Research 16: 154-156.

Sedgley M, Griffin AR (1989) Sexual Reproduction of Tree Crops. Academic Press Limited, pp. 53-127.

Spalik K (1991) On evolution of andromonoecy and ‘overproduction’ of flowers: a resource allocation model. Biological Journal of Linnean Society 42: 325–336.

Sharma CM, Khanduri VP (2007) Vegetative and reproductive phenophases in Aesculus indica Colebr. at two different altitudes in Himalayan forests. Current Science 92: 216-225.

Sternberg G, Wilson J (1995) Landscaping with native trees. Chapters Publishing. Shelburne, Vt.

Suo Z, Hashizume H, Yamamoto F (1995) Flower-bearing habits, pollen production, and pollen germination of Aesculus turbinata. Journal of Japanese Forestry Society 77: 535-544.

Stanley RG, Kirby EG (1973) Shedding of pollen and seeds. In: Kozlowski, T. (Ed.), Shedding of Plant Parts., pp. 295–340.

Stephenson AG (1981) Flower and fruit abortion: proximate causes and ultimate functions. Annual Review of Ecology and Systematics 12: 253–279.

Taiz L, Zeiger E (1998) Plant physiology (2nd ed.). Sunderland, MA: Sinauer Associates.

Troup RS (1921) Silviculture of Indian Trees: Vol. I. Oxford at the Clarendon, England.

Tuinstra MR, Wedel J (2000) Estimation of pollen viability in grain sorghum. Crop Science 40: 968-970.

Tupy J (1960) Sugar absorption, callose formation and the growth rate of pollen tubes. Biologia Plantarum 2: 169-180.

Yamasaki E, Sakai S (2013) Wind and insect pollination (ambophily) of Mallotus spp. (Euphorbiaceae) in tropical and temperate forests. Australian Journal of Botany 61: 60–66.

Tables

Table 1 Anthesis in relation with the time of the day in *A. indica*. TF: Total number of flowers opened, *n*= number of focal trees, and *sd*: slandered deviation

Time	<i>n</i> =3	
(h)	TF (mean ± sd)	% of anthesis
06:00	1498 ± 45.36	51.58 ± 1.35
08:00	402 ± 22.37	13.82 ± 0.43
10:00	262 ± 37.95	8.99 ± 1.08
12:00	333 ± 6.37	11.46 ± 0.18
14:00	253 ± 5.88	8.7 ± 0.26
16:00	157 ± 15.62	5.42 ± 0.38

Table 2 ANOVA of the effect of time and tree to tree difference on anthesis, the effect of time and concentration of solution on pollen germination, the effect of crown layers on (production of inflorescence, number of flowers, fruit set per inflorescence and reproductive success), and effect of pollination treatment on fruit set. *Significance at $P < 0.05$, NS= Non-significant.

Measured variable	Independent factor	df	F-value	P-Value ($P < 0.05$)
Flower opening	1. Sample trees	2	3.26 ^{NS}	0.81274
	2. Time	5	968.55*	0.00001
Pollen germination (perfect flower)	1. Time	5	48.74*	0.00001
	2. Concentration of solution	2	18.108*	0.00047
Pollen germination (Staminate flower)	1. Time	5	67.847**	0.00001
	2. Concentration of solution	2	31.130**	0.00005
Production of inflorescence	Crown layers	2	1.323 ^{NS}	0.33405
Number of flowers	Crown layers	2	1.272 ^{NS}	0.34618
Fruit set/inflorescence	Crown layers	2	12.239*	0.00763
Reproductive success	Crown layers	2	14.2377*	0.00527
Fruit set	Pollination treatments	3	13.8333*	0.01566

Table 3 Insect visitors to *A. indica* recorded at crop improvement research station, College of Forestry, Ranichauri, Uttarakhand, India during 8 observations in May.

Time (h)	Name of visitors	Scientific Name	Order	Number of individual	Duration of visit
08:00 - 08:00	Bumble Bee	<i>Bombus spp.</i>	Hymenoptera	22	13-15 sec
	Thrips		Thysanoptera	Infinite	
09:00 - 10:00	Bumble Bee	<i>Bombus spp.</i>	Hymenoptera	12	8-13 sec
	Honey Bee	<i>Apis mellifera</i>	Hymenoptera	7	4-8 sec
	Thrips		Thysanoptera	Infinite	
	Himalayan Spotted Flat	<i>Celaenorrhinus munda</i>	Lepidoptera	5	7-13 sec
10:00 - 12:00	Bengal Spotted Snow Flat	<i>Tagiades menaka</i>	Lepidoptera	4	6-16 sec
	Thrips		Thysanoptera	Infinite	
	Honey Bee	<i>Apis mellifera</i>	Hymenoptera	2	4-8 sec
12:00 - 14:00	Bengal Spotted Snow Flat	<i>Tagiades menaka</i>	Lepidoptera	2	6-16 sec
	Honey Bee	<i>Apis mellifera</i>	Hymenoptera	4	4-8 sec
	Thrips		Thysanoptera	Infinite	
14:00 - 16:00	Bengal Spotted Snow Flat	<i>Tagiades menaka</i>	Lepidoptera	3	6-16 sec
	Bumble Bee	<i>Bombus spp.</i>	Hymenoptera	9	8-13 sec
	Thrips		Thysanoptera	Infinite	
	Honey Bee	<i>Apis mellifera</i>	Hymenoptera	4	4-8 sec
	Humming Bird Moth	<i>Hemaris spp.</i>	Lepidoptera	2	1-2 sec
16:00 - 18:00	Bengal Spotted Snow Flat	<i>Tagiades menaka</i>	Lepidoptera	10	16 sec

Table 4 Reproductive success on different crown layers in sample trees of *A. Indica*. LC: Lower canopy, MC: Middle canopy, and UC: Upper canopy, (m ± mean ± standard deviation)

Attributes	<i>n</i> =3		
	LC (m ± sd)	MC (m ± sd)	UC (m ± sd)
Total number of inflorescence	43.6 ± 25.6	81 ± 48.5	16.6 ± 69.5
Total flower production	17672 ± 10451	32835 ± 19791	51270 ± 28877
Total number of fruit/ inflorescence	2.26 ± 1.9	2.9 ± 0.4	3.5 ± 0.3
Total number of fruit/ tree	103 ± 63	255 ± 162	464.6 ± 261
% of fruit set	0.58 ± 0.04%	0.74 ± 0.07%	0.93 ± 0.81
Average weight of fruit (g)		29.3 ± 0.5	
Total weight of fruit/ tree (g)	3012 ± 1834	7396 ± 4667	10560 ± 5467
Average number of ovules/ ovary		6	
Average number of seed/ fruit		1	
Average number of seed/ tree		769 ± 461	
Reproductive success	0.0058	0.0074	0.0093
Reproductive potential		0.167	

Figures

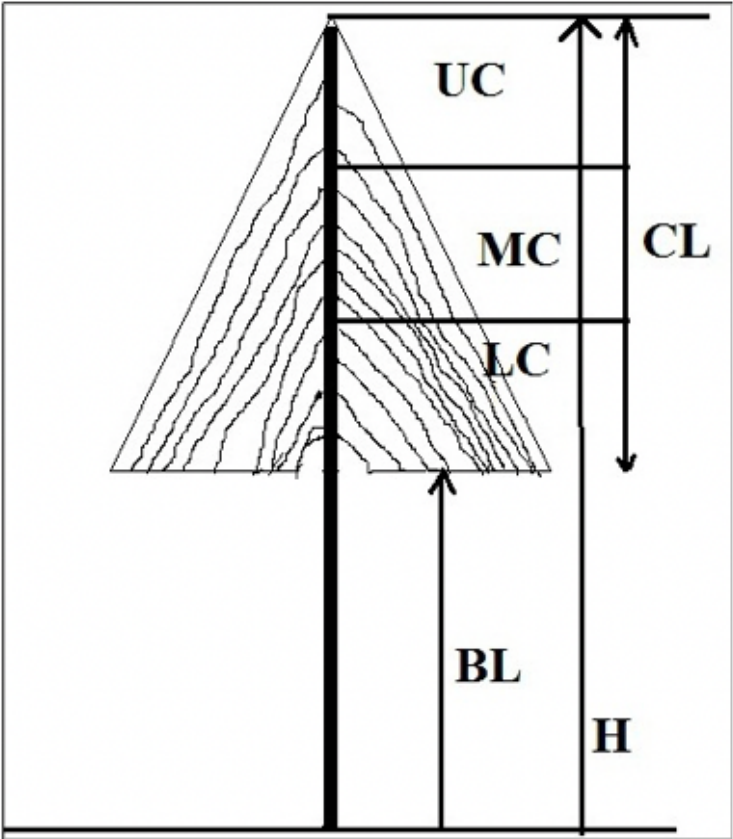


Figure 1

Diagrammatic sketch of crown differentiation for assessing reproductive success in crown layers. H: total height of the tree, BL: Bole length, CL: Crown length (H-BL), UC: Upper crown layer, MC: middle crown layer and LC: lower crown layer

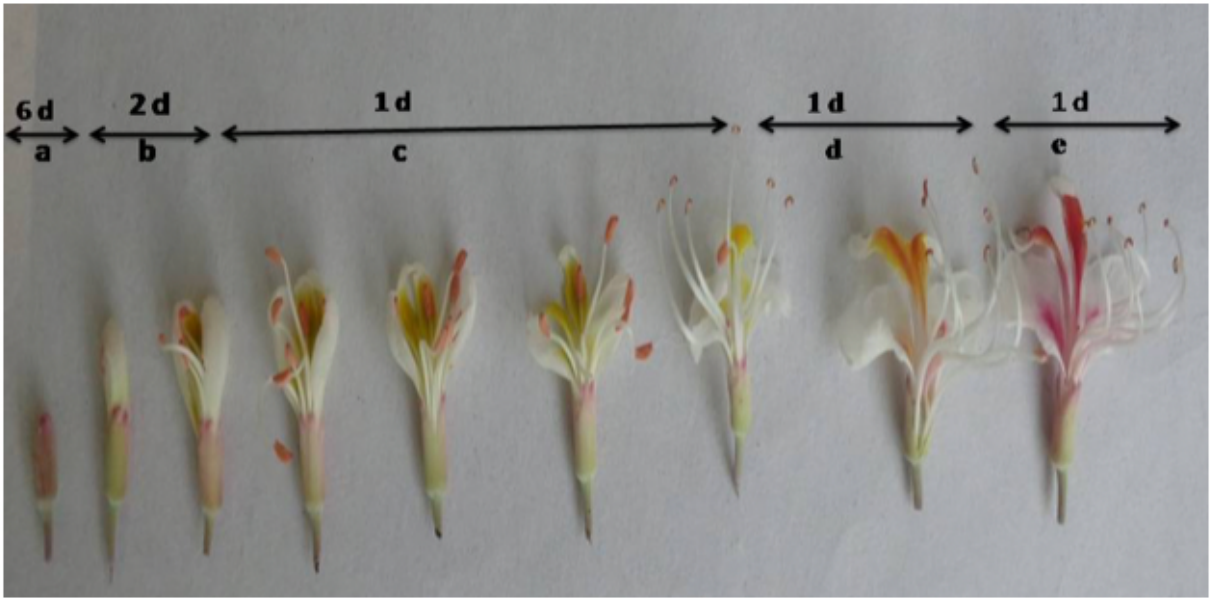


Figure 2

Individual flower developmental stages of *A. indica* and mean number of days to reach each flower stage. The main stages represented in sequence are from left to right, a: early bud to petal enlargement, b: anthesis, (c and d): pre-fertilization stages, d: post-fertilization stages