

Reproductive biology of *Acer caesium* Wall. ex Brandis (Sapindaceae): a dioecious tree species from Northwestern Himalayas

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Abstract

Acer caesium, commonly known as Himalayan maple, is a tree species endemic to Central and Western Himalaya. This multipurpose tree species has undergone a decline in its natural population number due to overexploitation over the years. Any attempts for conservation of this species will require understanding its reproductive behaviour in natural ecosystems. However, there is hardly any information on pollination ecology of this species. To bridge this gap, we studied the floral biology and pollination ecology in natural populations growing at Kedarnath Wildlife Sanctuary, Uttarakhand, India. In addition, whether the species is experiencing any pollen limitation was examined using hand-pollination experiments. *A. caesium* is a dioecious tree species with male biased sex ratio in the studied populations. Both male and female flowers exhibit typical characters of a wind pollinated plant. Interestingly, pollination experiments revealed ambophily in the species, with wind as the primary means of pollination and a small percentage of fruit set through insects. Pollen limitation experiments showed that natural fruit set in *A. caesium* is pollen limited as pollen supplementation augmented the fruit set. The probable reason for low fruit set in natural pollination conditions seems to be sparse distribution of the male plants around female plants in the population. Thus, for optimal conservation efforts, it is recommended that spatial structure of male and female individuals in the population must be considered for better outcrossing opportunities.

Introduction

Acer L., the maple genus, includes approximately 124 species (Renner 2007; Gibbs and Chen 2009) distributed in the northern hemisphere except, *Acer laurinum* Hassk., which is confined to the islands of Sulawesi and Java in Indonesia (Irwanto et al. 2020). *Acer* together with *Dipteronia* is placed under the tribe Aceraceae in the sub-family Hippocastanoideae of Sapindaceae (APG IV). With two-third of the species in the region, Eastern Asia is considered as the centre of origin for the genus and China is the major centre of diversity (Wolfe and Tanai 1987; Gibbs and Chen 2009). Maples are one of the most prevalent species of broad-leaved deciduous forests of temperate regions, often, acting as the keystone species of the community (Frelich et al. 2015). Rapid growth, wider adaptability, striking leaf shape and spectacular autumn foliage colours make them economically valuable as ornamentals for landscaping purposes (Saeki and Murakami 2009).

Genus *Acer* displays a great variation in the structure of its flower and sexual strategies. The sexual system in the taxon varies from monoecious (dichogamous, duodichogamous, heterodichogamous) to sex labile (andromonoecious, androdioecious) to strictly dioecious (De Jong 1976; De Jong 1994; Yadav et al. 2016). With such extensive variation in sexual strategies, genus *Acer* presents a remarkable opportunity to study the evolution of sexual systems and understanding the significance of reproductive traits in evolution of the genus. Although, the biogeographic history and role of *Acer* in the evolution of north temperate forest has received substantial attention not many studies have investigated the reproductive biology in the genus. This is mainly because of the inaccessibility of flowers due to large size of trees, short flowering season and habitat in difficult topographies. Nonetheless, few species that

have been worked out for their reproductive traits which include *Acer pycnanthum* (Saeki 2008), *A. pennsylvanicum*, *A. spicatum* (Sullivan 1983), *A. rubrum* (Sakai 1990) and *A. oblongum* (Yadav et al. 2019). Other than these accounts, studies related to the floral biology and morphology (Khushalani 1963; Yadav et al. 2016; Rosado 2018), sex expression and gender variation (Sato 2002), stigma biology (Peck and Lersten 1991) and microsporogenesis (Jacob and Lersten 1994) have also been carried out. Studies on the reproductive biology of *Acer* will be of interest in not only understanding the evolution of the genus but will also be helpful in predicting the consequences of human disturbance in forested ecosystems and devising conservation plans if the need arises

Acer caesium, commonly known as the Himalayan maple, is endemic to Central and Western Himalaya. It is sparsely distributed from Kashmir to Nepal between 2,000 and 3,000 m above sea level (asl) and some isolated trees have also been reported from coniferous forests up to 3,800 m in Kashmir and Pakistan (Singh and Kachroo 1976; Flora of Pakistan, eFlorae, 2009). Within India natural distribution of *Acer caesium* is reported from Himachal Pradesh, Uttarakhand and Jammu and Kashmir. Himalayan maple is the largest maple in the Western Himalayas growing up to 30 m in height and is a characteristic tree of the moist temperate deciduous forests of the Western Himalaya. Like other maple species, Himalayan maple plays a key role in forest ecosystems. The species is ecologically important in the temperate forests of the Western Himalaya for the watershed protection of mountain slopes. However, once a very common tree in the Western Himalaya, *A. caesium* became threatened due to overexploitation for both industrial and nonindustrial products, leading to its inclusion in the Red Data Book of Indian Plants (Nayar and Sastry 1987). Notably, the species is currently placed as 'Least Concern' by the IUCN (<http://www.redlist.org>) which seems to need revisitation as indicated by our exploratory surveys and a recent survey in northwestern Himalayas by Suman et al. (2022). Populations of Himalayan maple is declining in the surveyed areas emphasizing the need for its conservation in the region.

The present study aims at studying the reproductive biology of *A. caesium*. Very little information is available on sexual system in *A. caesium*. Survey of literature shows only two reports, one mentions it as monoecious but "poorly known" (Van Gelderen et al. 1994) and the other one deduces it to be heterodichogamous and androdioecious (Gleiser and Verdú 2005). Notably, an extensive phylogenetic analysis by Renner et al. (2007) to deduce evolution of dioecy in the genus could not include data from this species because of lack of information. Similarly, information on the phenology, floral biology, pollination biology in *A. caesium* is also cursory and inconclusive. With the aim to bridge this gap, we examined the pollination and breeding system of the species in its natural habitat. The study also addresses, how population thinning influences reproduction in *A. caesium*. Understanding these aspects will be critical for conservation of *A. caesium* in nature and also establishment of new plantations.

Material and Methods

Study species and site – *Acer caesium* is the largest of the maples in the Western Himalayas with tree height ranging between 10 to 25 m (Fig. 1a, b). It is a deciduous tree with grey bark, glabrous branchlets, 5-lobed (rarely 3-lobed) purplish green leaves possessing cordate base, serrulate margin and acuminate

apex. The circumference at breast height (CBH) is between 1.3 and 2.7 m. On the basis of the available literature on the natural distribution of the *A. caesium*, the Kedarnath Wildlife Sanctuary (KWS) of Uttarakhand, a state in northern part of India was selected for study. The KWS is one of the largest protected areas (975 km²) in the Central Western Himalaya. About 182 villages are situated around KWS, of which about 50 are located very close to the sanctuary. For the study two microsites were delimited (Kachula Kharak, KWS1 N 30°27'40" E 79°13'39.8", and Vasuki, KWS2 N 30°27'52.3" E 79°13'22.18"). The climate of the study area is typical moist temperate type, which receives moderate to high snowfall from December to February. Mean annual maximum temperature recorded during the study period was 16.41 ± 3.60°C, whereas mean annual minimum temperature was 6.14 ± 1.98°C. Mean Relative humidity round the year ranged from 15–86%.

Flowering sex ratio – Observations in the first year of our survey showed that *A. caesium* is a dioecious species. Accordingly, the sex of each reproductive tree was recorded (118 and 91 reproductive trees at KWS1 and KWS2) by observing floral morphology and fruiting status during the flowering and fruiting periods. Trees were carefully observed for three consecutive years to see any incidences of sex lability.

Floral biology – The morphological details of the male and female flowers were studied under a stereo-zoom microscope and documented photographically. The dimensions were measured using a digital vernier caliper. For scanning electron microscopy, the flowers were fixed in Karnovsky's fixative (4 h, 4°C) and washed twice with sodium cacodylate buffer (0.2 M). After which anthers and pistil were dissected from male and female flowers respectively and dehydrated in a graded series of acetone (10–100%, 30 min interval each), critical point dried (CPD), mounted on aluminium stubs with a double adhesive tape and coated with gold–palladium alloy for 25 s at 40 mA using Autofine Coater before observing under a scanning electron microscope (SEM) at 3–5 kV (Jeol, JSM-6610LV at University of Delhi).

To estimate the average number of pollen grains produced in a male flower, pollen suspension method was used (Dafni et al. 2005). For which, stamens from a ready-to-open flower (N = 25 flowers each population) were crushed in 1 ml of suspension solution (50% ethanol with 0.5% Triton-X, v/v) and vortexed for 1 min. Then 25 µl of the suspension was placed over a clean glass slide and a coverslip was gently lowered. The number of pollen grains was counted under the bright field microscope. The value obtained from 25 µl suspension was multiplied by a factor 40 to determine the total number of pollen grains produced by a flower. For assessment of pollen viability, pollen grains from freshly dehisced anthers (N = 10) were collected on a dry petri dish and stored in gelatin capsules. Samples were brought to the laboratory in a desiccator for further processing (Chanon and Jourdan 1998). Pollen viability was assessed using fluorochromatic reaction (FCR) test at 24, 48 and 72 h after anther dehiscence (maintained under laboratory conditions, Heslop-Harrison J and Heslop-Harrison Y 1970). The duration of stigma receptivity was estimated by peroxidase test (Dafni et al. 2005). Fresh unpollinated female flowers of three different developmental stages based on the sizes of their stigmas were used. The developmental stage with the most receptive stigma was used for manual/controlled pollination experiments.

Pollination ecology – To determine the possible role of biotic agents in pollination, flowering branches in both male (N = 10) and female (N = 10) trees suitable for observation were marked in each population. Selected inflorescences were observed with the help of binoculars and floral visitors were photographed using a DSLR camera (Nikon). Observations were staggered over periods of 30 min each hour from 0600 to 1800 h for a period of one week during the peak time of flowering. Documentation of the types of visitors, average time spent on each inflorescence, and foraging behaviour was carefully accomplished. Insect visitation was also recorded for first two nights with a hand-held battery torch. However, due to the absence of any nocturnal foragers, observations were subsequently confined only to day time.

To determine the contributions/role of wind and insects in pollination and seed set, different manipulations were used for vector exclusion from inflorescence (1) Inflorescence and buds were kept open to insects and wind (i.e., open-pollinated stigmas) (2) 1-mm-mesh bags were used to exclude insects but allow wind-borne pollen. Comparing (2) with (1) indicated the contribution of insect pollination, and (2) indicated the contribution of wind pollination.

Foraging behaviour and the flower handling time – The number of visits made by an insect and the average time spent by an insect on the flower were recorded with the help of a stop-watch. Landing on or touching a flower was considered a visit. The time interval between the successive movements of an insect within an inflorescence was taken as the time spent on a flower. Observations were taken in the intervals of 10 min in which the number of visits to a known number of inflorescences was carefully recorded. The number of inflorescences observed at a time was adjusted so that an accurate count can be obtained, with more inflorescences being watched when visitation rates were low and vice versa.

Pollen load on the foragers – The insect foragers were captured and collected (Dafni 1992; Kearns and Inouye 1993). To determine pollen load, the collected insects (N = 5) were analyzed for pollen deposition on their body parts, and the total amount of pollen load was determined following the methodology described by Dafni and Calder (1987).

Pollen limitation – To test whether fruit production in *A. caesium* was pollen limited, pollen supplementation experiments were done during 2012-14 at KWS1. For this, isolated female trees at KWS1 were divided into either open-pollinated control treatment or supplemental outcross pollen treatment (N = 5 each). In the control treatment, the inflorescences (N = 50) were tagged and left open for natural pollination to occur. In the other set up, trees were supplemented with a mixture of outcross pollen. Inflorescence (N = 50) used for the treatment were bagged a day prior to anthesis. Next day, flowers were randomly supplemented with a mixture of outcross pollen from a group of 5 donor plants that were at least 20 m or further from the recipient. Mixing pollen closely mimic natural pollination and also increases the diversity of pollen delivered (Ashman et al. 2004). After supplementation of pollen on to receptive stigmas of flowers, the inflorescences were bagged again. Bags were opened regularly to check the status of stigma until the flowers dropped. After a month from the date of pollination, bags were opened to check for fruit set. Percent fruit set in both the sets of experiments was compared to check for effect of pollen limitation on the pollination efficiency of plant.

Statistical analysis – The data was analyzed statistically using SPSS V 24 (IBM Corp 2016.) Descriptive statistics was used to calculate the means and standard deviations. Two-way ANOVA was performed for pollination manipulations and pollen limitation experiments. Different treatments used in pollen limitation were further investigated with Bonferroni corrected paired t-tests and the values were considered significant at $P < 0.05$.

Results

Sex ratio – At KWS1 out of 118 trees, 84 were male and 34 were female and at KWS2, 60 were male and 31 were female. The effective sex ratio of both the populations is significantly male biased (KWS1: proportion male = 0.71, $G = 21.186$, $p < .00001$; KWS2: 0.65, $G = 9.242$, $p = 0.00237$). We found no records of sex lability in the species during the course of our study.

Floral biology – *Acer caesium* is a dioecious species with male and female flowers borne on separate individuals. The peak time of flowering occurs during fourth week of March across the studied populations. The male and female flowers are borne in cymosely branched terminal corymb (Fig. 1c, d). Average number of inflorescences in a bunch is 11.36 ± 0.34 in the in the male tree and in female tree the number is 8.18 ± 0.52 . Average number of flowers in an inflorescence is 13.89 ± 1.23 to 10.34 ± 1.05 in male and female plants respectively (Table 1). Both male and female flowers are small, yellowish green, and possess floral structures that promote wind pollination (Fig. 1c–e). The emergence of one of the stamens or the stigma from the bracts indicated the initiation of anthesis. Both male and female flowers display peak anthesis between 1000–1200 hours.

Table 1
Floral biology of staminate and pistillate flowers of *A. caesium*

Characters	Staminate	Pistillate
Number of inflorescence in a bunch	11.36 ± 0.34	$8.18 \pm .52$
Number of flowers in an inflorescence	13.89 ± 1.23	10.34 ± 1.05
Size of flower (mm)	5.30 ± 0.18	5.46 ± 0.04
Length of stamens (mm)	5.24 ± 0.28	-
Size of pistil (mm)	-	2.13 ± 0.13
Number of anthers per flower	8–10	-
Number of pollen grains/ flower	43760 ± 1392	Nil
Number of ovules/ovary	-	02
Pollen: ovule ratio	21880:1	
Predicted breeding system	Obligate xenogamy	

Average size of the male flower is 5.30 ± 0.18 mm. Each male flower possesses 8–10 stamens and length of a mature stamen, exerted from the flower is 5.24 ± 0.28 mm (Fig. 1f). Pollen grains are produced in large number in the anthers and estimated to be 43760 ± 1392 in a mature flower. Mature anther opens through longitudinal slit to release yellow, smooth, dry, powdery and tricolporate pollen grains (Fig. 1g). Pollen grains lose their viability within 48 hours from the anther dehiscence. Female flower is represented by bicarpellate pistil (2.13 ± 0.13 mm) which is distinguishable into a subspherical ovary overtopped by a bifid stigma; the style is inconspicuous (Fig. 1h). The adaxial surface of stigma is covered with papillae whereas the abaxial surface is smooth (Fig. 1i). The inner papillate surface is recurved and degree of tabulation of papillae varies in different flowering stages. The stigma is most receptive at the time of anthesis and the receptivity gradually declines and becomes negligible after two days of anthesis. Ovary is bilocular with one ovule in each locule.

Pollination ecology – Observations show that *A. caesium* species is predominantly wind pollinated. Morphological features of flowers are also conducive to wind pollination. However, during day time different insect species visit the male and female inflorescences to collect pollen and contribute to additional fruit set. Pollen grains acts as the floral reward which were seen on the head and thorax region of the insects. Insect visitors have been described in detail in an earlier publication by us, Roy et al. (2017). Among the recorded species of insects, giant Asian honeybee (*Apis dorsata*) (Fig. 2) and Asian honeybee (*Apis cerena*) actively take part in pollination. *A. dorsata* showed a longer flower handling time and pollen load than *A. cerena* (Table 2). Head, wings, and hind limbs were the major body parts laden with pollen. Average natural fruit set in *A. caesium* at KWS1 and KWS2 was 32.39 ± 2.87 and 30.02 ± 3.86 respectively (Table 3). Assessment of contribution of the two agencies of pollination in the fruit set showed that wind is the major contributor and a small percentage is contributed by insects. In the insect-excluded bagged flowers, where wind was the only mode of pollination, the fruit set was found to be 27.06 ± 3.95 and 23.8 ± 3.87 in KWS1 and KWS2 respectively (Fig. 3, Table 3). Thus, there was an average decrease of 5.33 ± 1.65 (KWS1) and 6.22 ± 0.198 (KWS2) in the fruit set when the insects were excluded or in other words the contribution of insects in pollination.

Table 2
Pollen load and foraging period of various floral visitors of *A. caesium*

Floral visitor	Common Name	Foraging duration/ inflorescence (sec) (n = 20)	Pollen Load
KWS1			
<i>Apis dorsata</i>	Giant Asian honeybee	5.3 ± 1.8	292 ± 39
<i>Apis cerena indica</i>	Asian honey bee	3.6 ± 1.6	229 ± 22
KWS2			
<i>Apis dorsata</i>	Giant Asian honeybee	5.8 ± 2.1	271 ± 41
<i>Apis cerena indica</i>	Asian honey bee	3.9 ± 1.2	206 ± 36

Table 3
Details of fruit set in response to different pollination treatments in *A. caesium*

Treatment	Percent fruit set					
	POP KWS1			POP KWS2		
	2012	2013	2014	2012	2013	2014
Open pollination (Wind + bees)	29.12 (172/590)	33.56 (205/614)	34.51 (187/541)	26.63 (167/627)	29.22 (186/633)	34.23 (206/601)
Bees Excluded	22.59 (142/629)	30.12 (174/576)	28.48 (183/640)	20.25 (127/627)	23.22 (147/633)	27.94 (171/612)
Values in parentheses refer to the number of fruits formed/ number of flowers bagged for pollination studies						

Pollen supplementation in the KWS1 population of *A. caesium* shows an increase in the percent fruit set compared to controls (Fig. 4). Open pollination yielded an average of 33.60 ± 2.08 percent fruit set whereas pollination with supplemented outcross pollen yielded 52.27 ± 2.22 percent fruit set respectively. Two-way ANOVA showed that fruit-set following pollen supplementation was significantly greater than open pollination ($F = 1752.156$, d.f. = 1, 15, $P = 0.0003$).

Discussion

Male biased sex ratio – The effective sex ratio of flowering *A. caesium* trees at the studied populations was male-biased. This finding is in line with many woody dioecious tree species (Queenborough et al. 2013; Sinclair et al. 2012; Matsushita et al. 2016). Even many maple species exhibit male biased sex ratio

especially under xeric conditions like *A. campestre*, *A. pensylvanicum*, *A. negundo*, *A. saccharinum*, *A. rubrum* (Hibbs and Fischer 1979; Sakai 1990). Male biased sex ratio in tree species is believed to be due to difference in reproductive cost incurred by the two sexes. Usually, females invest more than males on reproduction and less on growth and maintenance and males tend to flower more frequently and flower at smaller sizes than females (Obeso 2002). Thereby, females tend to have low survival rates than males under xeric habitats.

Sexual system – Sexual system in the genus *Acer* varies from monoecious (dichogamous, duodichogamous, heterodichogamous) to sex labile (andromonoecious, androdioecious) to strictly dioecious (De Jong 1976; De Jong 1994). From the present study it can be clearly concluded that the sexual system in *A. caesium* is dioecious. Dioecism is known in thirteen species of *Acer* other than *A. caesium* and is reported to have evolved in three clades of the genus (Van Gelderen et al. 1994; Renner et al. 2007). Ancestral sexual system of *Acer* is hypothesized to be monoecy with dichogamous or duodichogamous flowering (De Jong 1976; Renner et al. 2007). However, according to Gleiser and Verdú (2005) the ancestral state in *Acer* was heterodichogamous androdioecy. Evolution of dioecism by monoecious pathway is usually associated with variation in sex expression and sex lability (Geber et al. 1999; Freeman et al. 1997) such as in *Mercurialis annua*. However, extensive survey of the natural populations of *A. caesium* at study sites did not reveal any sexual polymorphism in the species, and also no sex lability was observed in the populations.

Floral morphology and pollination – Morphologically, the floral features of *A. caesium* are compatible with wind pollination. It produces large number of small, smooth and dry pollen grains that can easily be transported by wind. Reduced investment in the non-reproductive parts of flower and resources like fragrance or nectar, maximizes resource allocation to pollen production. Other adaptive features for anemophily include flowering in leafless canopies, pendulous nature of the inflorescence, stigmas with papillae that increase surface area, and synchronized flowering. Like other woody, perennial wind pollinated species, *A. caesium* exhibits high pollen to ovule ratio. Abundant pollen production and reduction in the number of ovules in wind pollinated plants ensures fertilization under untargeted pollen flow (Friedman and Barrett 2011).

Although floral characters of *A. caesium* are seemingly indicative of wind pollination, our pollination experiments showed that insect pollination also contributes to fruit set. The present study shows that *A. caesium* is ambophilous, with major contribution by wind and small percentage of fruit set by insects. Unlike wind for which specialized floral suites are present in the species, there are no floral features adaptive for insect pollination seen in the species. However, mass floral display seems to be an attractant for the insects. As the species flowers in the early spring when flowering in the region is still to peak, pollen produced by species act as a source of critical nutrients for pollinators during an otherwise period of poor pollen availability.

Flowers of *A. caesium* are visited by a variety of insect belonging to different taxonomic groups (Roy et al. 2017). Insects visit both male and female flowers with equal frequency and peak frequency of

visitations to male and female flowers occurs during the same time in a day. However, pollination is accomplished by two species of bees, *Apis dorsata* and *A. cerena*. Generalist pollinators like bees have few or no specialized adaptations for flowers and indiscriminately visit any plant that is flowering in an area. Pollination by small, generalist insects is common in dioecious plants even though the female flowers do not offer pollen or any other reward (Thomson and Brunet 1990; Sakai and Weller 1999). Charlesworth (1993) proposed that these generalist pollinators lack discrimination between male and female flowers, enabling the evolution of small, unattractive female flowers by reducing the required resource allocation to attractive structures. Biosystematics studies by De Jong (1976) show that most of the maple species are still evolving and considering the mode of pollination of *A. caesium*, we believe that this tree species is in last stages of transition from insect pollination towards complete wind pollination. Results of pollination mechanism in *A. caesium* also indicate inbreeding avoidance and sexual specialization to be the selective forces driving the evolution of dioecy in the genus.

Pollen limitation – Occurrence of pollen limitation should fundamentally be rare in wind-pollinated plants as they produce abundant pollen grains (Culley et al. 2002). Pollen limitation is rather mostly associated with insect-pollinated plants. There are numerous studies in insect-pollinated plants which show pollen limitation leads to either reduced seed yields or increased incidences of selfing (Murawski and Hamrick 1991; Murawski and Hamrick 1992; Ashman et al. 2004; Larson and Barrett 2000, De Jong et al. 2005; Roy et al. 2021). Such studies are rare and few in wind pollinated plants. Experimental evidences in the present study suggest that *A. caesium* experiences pollen limitation at the study sites. Pollen limitation in wind pollinated plants is usually a consequence of decreased density of conspecifics such that pollen quantity may be inadequate for potential seed set. Several studies have reported pollen limitation in wind pollinated plants as a result of small population sizes or fragmentation (Knapp et al. 2001; Wilcock and Neiland 2002; Davis et al. 2004; Steven and Waller 2007; Hesse and Pannell 2011; Roy et al. 2021). In fragmented landscapes, pollination rates can be influenced by population density and dioecious plants are expected to be more sensitive to changes in population size and structure. Although females in a male biased population like in *A. caesium* should not be expected to experience pollen limitation but the structure of population will have a major impact on the amount and quality of pollen captured by stigmas. Thus, the average distances between trees in dioecious species is very crucial as increased distance from the males leads to decreased fruit set due to pollination limitation. For instance, in dioecious *Thalictrum fendleri*, *T. dioicum* and *Rumex nivalis* increased distance from males led to reduced seed set (Steven and Waller 2007; Stehlik et al. 2008).

The fact that fragmentation can induce pollen limitation is established but there are also few studies which show that pollen limitation due to fragmentation can lead to a biased sex ratio in dioecious plants (Taylor et al. 1999; Stehlik et al. 2008; Yu and Wu 2011). For instance, in dioecious herb *Silene alba*, sex ratio was shown to be directly influenced by the quantity of pollen. A mixture of pollen from several males could produce more female progeny than pollen from a single male (Taylor et al. 1999). This shows that the amount of pollen captured by stigmas can potentially affect the sex of the offspring. Similar studies in the *A. caesium* populations can provide stronger evidence of the influence of fragmentation on sex ratio. Himalayan maple experiences significant pollen limitation as the natural populations are small and

the distances between male and female trees are large for effective pollination by wind. This hinders *A. caesium* from realizing its reproductive potential which can have negative consequences for the species in the long run. In view of the above findings, it is proposed that in future if conservation plans are undertaken for the species, spatial structure of male and female individuals in the population should be given priority for the conservation of this dioecious plant species.

Declarations

The authors have no financial or proprietary interests in any material discussed in this article.

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Author contribution SKR conducted fieldwork, gathered and analyzed macro- and micromorphological data and contributed to analyses, writing, and discussion of manuscript. PK contributed to analyses, writing and discussion of manuscript. AKB and AKP provided research funds, supervised the investigation and led writing, and discussion of manuscript.

Conflict of Interest Statement There is no conflict of interest among the authors.

Data Availability Statement The datasets generated during and/or analysed during the current study are available with the corresponding author on reasonable request.

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Figures

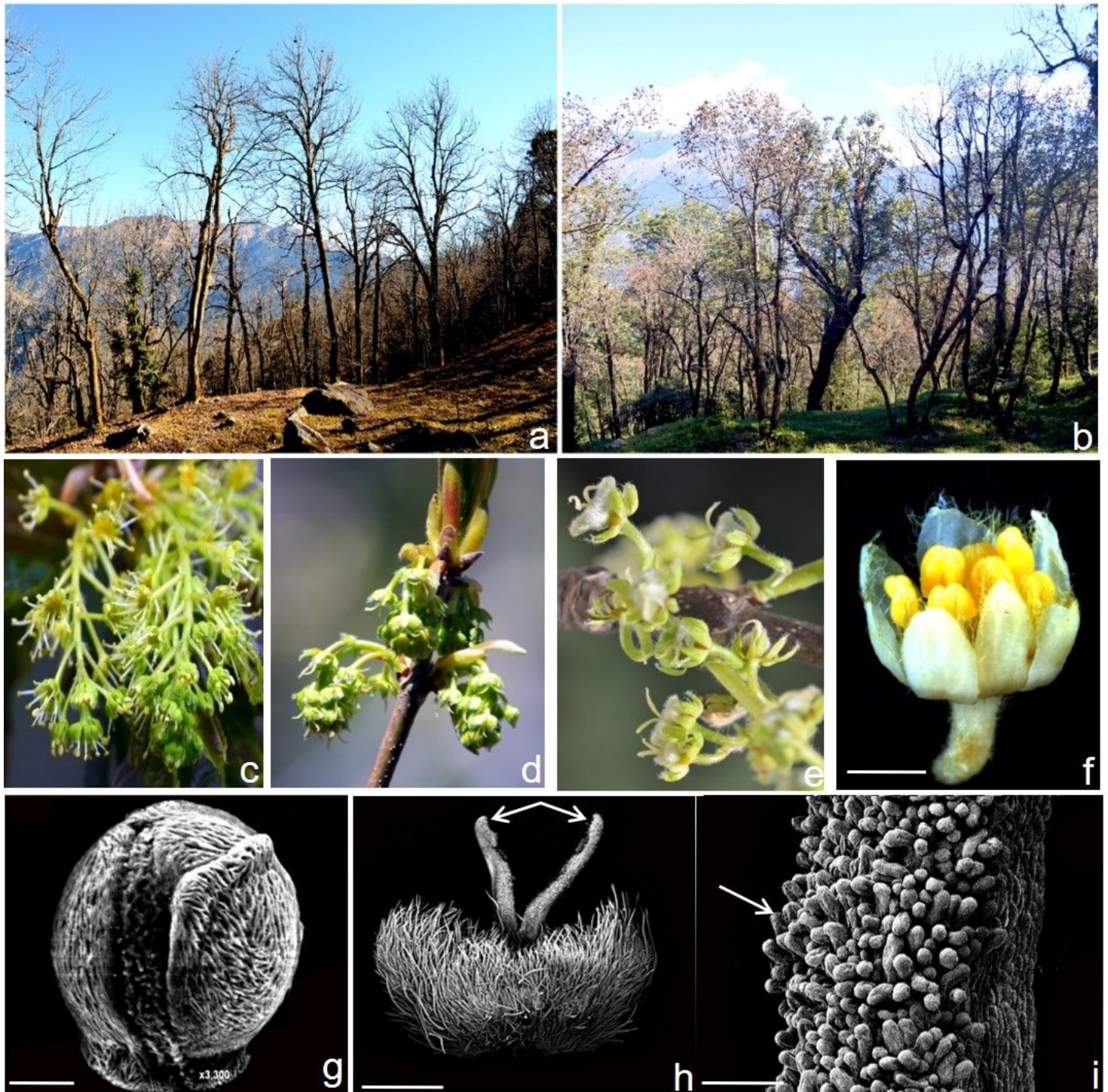


Figure 1

(a, b) Populations of *Acer caesium* at Kedarnath Wildlife Sanctuary of Uttarakhand; (c) male inflorescence; (d) female inflorescence; (e) female flowers; (f) a male flower; (g) SEM of a pollen grain; (h) SEM of a pistil showing papillate stigma (arrows) and ovary; (i) details of stigma papillae. Scale bars: f: 1.3 mm; g: 5 μ m; h: 1.5 mm; i: 100 μ m



Figure 2

(a, b) *Apis dorsata* foraging on male and female flowers

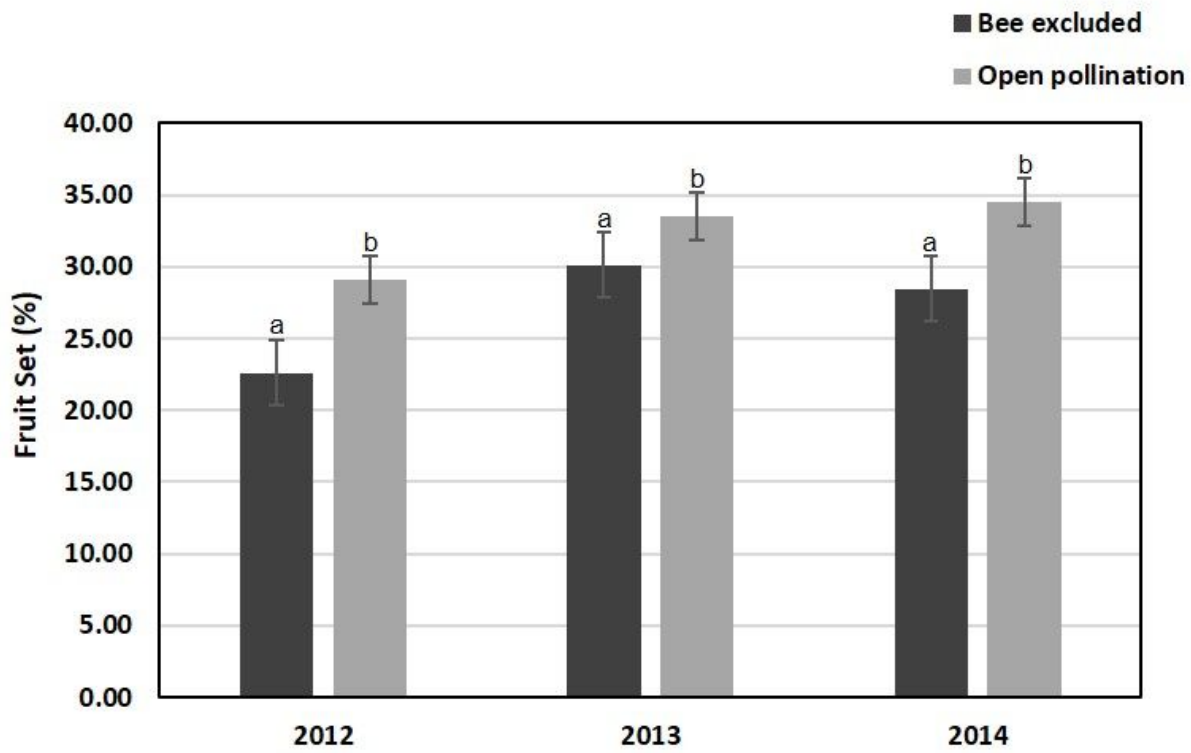


Figure 3

Bees exclusion experiment at KWS1. Error bars represent the standard error of the mean (SEM). Different letters indicate significant differences ($P < 0.05$)

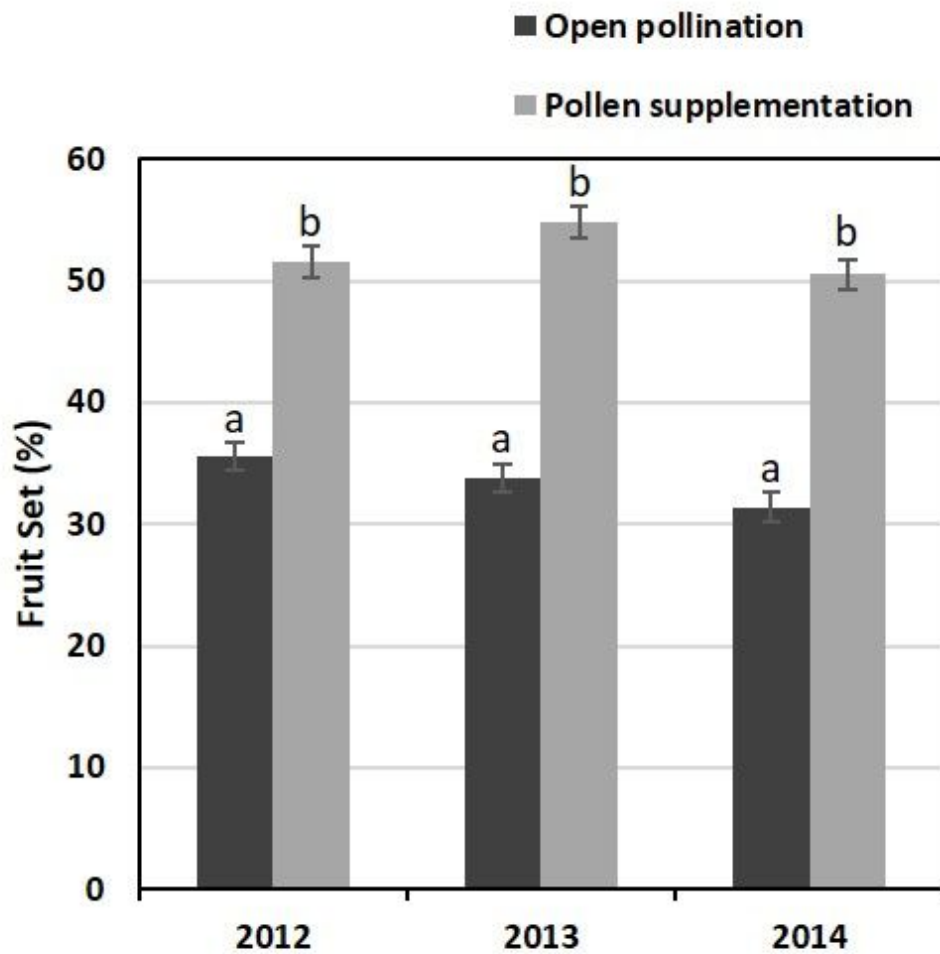


Figure 4

Comparison of female reproductive success in terms of percent fruit set in open-pollinated and pollen-supplemented plants at KWS1. Error bars represent the standard error of the mean (SEM). Different letters indicate significant differences ($P < 0.01$)