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Cryptic monoecy and floral morph types in *Acer oblongum* (Sapindaceae): An endangered taxon

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A B S T R A C T

Maple trees are known for their varied sexual and floral systems. One such system, andromonoecy, has generated considerable interest in terms of sexual system and evolution in angiosperms. In *Acer oblongum* three different flowering morphs were present on each individual: Staminate type I (Male type I), hermaphrodite and Staminate type II (Male type II) flowers. Staminate type I flowers bloom early in the season followed by hermaphrodite and Staminate type II. The objective of this study was to elucidate sexual system in *A. oblongum* by comparing male and female organ performance of different floral morphs and assess its adaptive significance. Phenology, morphometry, resource allocation patterns, and pollen and pistil performance of three morphs were compared in order to elucidate the sexual system. Significant differences were observed in resource allocation patterns of different floral morphs. The variation was mainly because of androecium and gynoecium weight. Anthers of hermaphrodite morphs remain indehiscent while number and size of anthers were less than that of staminate types. The number of pollen per flower in case of hermaphrodite flowers was smaller and their pollen showed lower viability when compared with staminate types. Although pollen from hermaphrodite flowers failed to germinate on the receptive stigmas, it yielded positive FCR viability test. Morphologically hermaphrodite flowers of *A. oblongum* do not possess male function and are functionally female, although a relict function of attracting pollinators cannot be ignored. *Acer oblongum* is cryptically monoecious while functionally andromonoecious.

1. Introduction

Andromonoecy is the occurrence of male and hermaphrodite (perfect) flowers on the same individual. Either flowering morphs may be present simultaneously or one after the other, on the same individual, influenced by the change in temperature conditions (De Jong, 1976). Andromonoecy is widely distributed among major angiosperm taxa in approximately 4000 species representing 33 families (Yampolsky and Yampolsky, 1922; Bawa and Beach, 1981; Cruden and Lloyd, 1995; Miller and Diggle, 2003; Vallejo-Marin and Rausher, 2007a). Evolution of andromonoecy has been from hermaphroditism by loss of female structure (pistil) in staminate flowers, and is considered as the first step towards evolution of monoecy, androdioecy and dioecy (Primack and Lloyd, 1980; Bertin, 1982). The evolution of andromonoecy has attracted considerable attention of reproductive and evolutionary biologists (Narbona et al., 2002; Connolly and Anderson, 2003; Huang, 2003; Cuevas and Polito, 2004; Schlessman et al., 2004; Perglova et al., 2006; Tanaka et al., 2006; Miller and Diggle, 2007; Vallejo-Marin and Rausher, 2007a,b). Three main hypotheses have been proposed regarding evolution of staminate flowers and andromonoecy: (1) The optimal resource allocation hypothesis proposes that the production of male flowers reduces resource investment and permits the resources saved to be re-allocated towards other fitness-enhancing traits (Bertin, 1982; Emms, 1993; Liao et al., 2006; Vallejo-Marin and Rausher, 2007b; Kouonon et al., 2009). (2) The increased pollen donation, in contrast sets forth that male flowers are more efficient in donating pollen than hermaphroditic flowers for any of the following reasons – more or larger pollen, higher pollen viability or greater attractiveness to pollinators by increased floral display and larger anther size (Charlesworth and Morgan, 1991; Harder and Barrett, 1996; Barrett, 2002; Kouonon et al., 2009). (3) Implies an increased pollen receipt on hermaphroditic flowers which also predicts greater seed production by plants, thereby increasing female reproductive success (Sunnichan et al., 2004; Vallejo-Marin and Rausher, 2007b; Vary et al., 2011; Shivanna and Tandon, 2014).

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An important feature of andromonoecy is its role in cross-pollination (Kouonon et al., 2009). In general, outcrossed progeny has higher levels of genetic diversity and vigour than those produced by selfing, because of the effect of inbreeding depression and expression of deleterious recessive alleles in latter (Schemske and Lande, 1985; Charlesworth and Charlesworth, 1987). On the contrary, selfing provides reproductive assurance when the pollinators are scarce or limited (Holsinger, 2000). Inbreeding depression can play an important role in the evolution of mating systems, as reduced fitness of inbred progeny in comparison to outbred progeny is common in flowering plants (Charlesworth and Charlesworth, 1987; Husband and Schemske, 1995; Porcher and Lande, 2005).

The objectives of present study are: (1) to unveil the sexual system of *A. oblongum* by ascertaining the functional sex of three flowering morphs through analyses of pollen and pistil functions and phenological observations; (2) to study the “cost” of investment in flowers through resource allocation pattern along with morphometric investigations.

2. Materials and methods

Acer oblongum Wall. ex DC. (Sapindaceae) is a semi-deciduous tree, 15–18 m tall with its natural distribution in India, China, Nepal and Pakistan (Nayar and Datta, 1982). In India, *A. oblongum* occurs mainly in Arunachal Pradesh, Himachal Pradesh, Jammu and Kashmir, Meghalaya, Manipur, Nagaland, Sikkim, and Uttarakhand. *Acer oblongum* yields timber of commercial value and the leaves are used as fodder. Co-existing species of *A. oblongum* at the study sites in Central Himalaya are: *Fraxinus micrantha* Lingelsh., *Pinus roxburghii* Sarg., *P. wallichiana* A.B.Jacks., *Pittosporum eriocarpum* Royle, *Quercus floribunda* Wall., *Q. semecarpifolia* Sm. and *Rhododendron arboretum* Sm. *Acer oblongum* blooms in early spring (at the end of February) and flowering lasts for four to six weeks. Floral induction in *A. oblongum* occurs during early winters. However, the floral buds are not evident until February.

The study sites (Dehradun: 30°20′N, 077°59′E; Mussoorie: 30°26′N, 078°05′E; and Nainital: 29°22′N, 079°27′E) are located in Uttarakhand state (Central Himalaya), India, and the tree species is cited as Endangered in Red Data Book of Indian Plants (Nayar and Shastry, 1987). For phenological studies, 20 trees each were marked at different sites of Dehradun, Mussoorie and Nainital, and observed for three consecutive years (2013–2015).

2.1. Floral biology and phenology

The general sequence of pheno-events such as leaf fall, flowering, peak time of flowering, fruiting and seed dispersal, were regularly monitored by using a binocular. The flowering branches were accessed by using a collapsible aluminium ladder (9 m). A total of 40 inflorescences from five trees were sampled, their flowers were counted and longevity was recorded.

Three floral morphs viz. Staminate type I, hermaphrodite and Staminate type II were found on every individual of a population. Since there was a temporal separation of flowering between different floral morphs, the ratio of different types of morphs was calculated. The average number of each floral type born on an inflorescence was calculated. Dimensions of floral traits of three different morphs were determined using a digital Vernier Caliper.

2.2. Resource allocation pattern

A total of 25 flowers of each type was collected at bloom just before anther dehiscence, dissected into stamens, pistil and perianth, and dried at 60 °C for 72 h (Podolsky, 1993). Floral organ biomass has been used to compare the “cost” of investment in flowers within species (Primack and Lloyd, 1980; Bell, 1985) and across mating systems (Cruden and Lyon, 1985). The relationship between fresh and dry weight of flowers was determined using one-way ANOVAs (Cuevas and Polito, 2004). Different measurements of floral organs (length, width and diameter) were made using a caliper on each type of flowering morph (Zhang and Tan, 2009). Differences between the three floral types were calculated using one-way ANOVAs.

2.3. Pollen and pistil performance

Staminate type I, hermaphrodite and Staminate type II flowers were collected and their anthers were allowed to dehisce at room temperature. The pollen was collected and stored at room temperature. Pollen grain number was calculated for 20 flowers of each type following the method of Cruden (1977) under a brightfield microscope using a graduated coverslip. Pollen viability was ascertained by fluorochromatic reaction (FCR) test (Heslop-Harrison and Heslop-Harrison, 1970; Shivanna and Rangaswamy, 1992) on at least 200 pollen grains for each pollen type. Size of pollen grains was determined following the method of Hrycan and Davis (2005). Pollen grain size of each type was calculated for 100 hydrated pollen grains using a calibrated stage and an ocular micrometer. Pollen characteristics (shape, size, exine pattern) for each pollen type were studied using SEM (scanning electron microscopy). Stigma type, whether papillate or not, of the three morphs was also ascertained using SEM, and estimation of stigma receptivity was made using Benzidine and H_2O_2 (Dafni, 1992).

3. Results

3.1. Floral biology and phenology

Acer oblongum flowers during the end of winter, from late February to early April. Earliest to appear on a tree are Staminate type I flowers followed a week later by buds of hermaphrodite flowers and Staminate type II flowers. The Staminate type II flowers open a little later than the hermaphrodite flowers when the stigma of the latter becomes receptive. The flowers are actinomorphic, hypogynous with five green linear or lanceolate sepals and five greenish-white elliptic-lanceolate petals. The flowers are borne terminally in corymbose inflorescences and are odourless and nectarless. Three types of flowers were observed on the same inflorescence at different times. There was a temporal difference of almost a week between the blooming of three types of flowers. Therefore, the proportion of different flowering morphs on an inflorescence (N = 20) was calculated and it was found that early blooming Staminate type I flowers accounted for the highest proportion at approximately 72%, followed by hermaphrodite flowers (15–16%), while Staminate type II (11–13%) flowers had the lowest share (Fig. 1). Staminate type I flowers (first peak in pollen availability) start blooming in the last week of February, followed by hermaphrodite flowers more than a week later. Staminate type II flowers appear (second peak in pollen availability) approximately 4–5 days after the peak blooming of morphologically hermaphrodite flowers in the third week of March (Fig. 2) when the ambient temperature increases. The receptivity of stigma of hermaphrodite flowers coincides with dehiscence of the anthers of Staminate type II flowers. Pheno-events such as leaf fall, peak time of flowering, fruiting have been recorded in trees growing in natural habitat (Figs. 2, 3A–D).

Staminate type I flowers are pentamerous, pedicellate, hypogynous, morphologically perfect (rudimentary pistil is present), with 9–11 anthers present in two whorls. The outer whorl of anthers