

Pollination Ecology of *Impatiens rufescens* (Balsamineae) - An Endemic Annual Herb From Nilgiri Mountains, Western Ghats, India

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ABSTRACT

I examined the breeding and pollination systems of *Impatiens rufescens* (Balsamineae), a rare annual balsam restricted to the Western Ghats of India. The experiments were conducted in the Korakundah (KR) and Upper Bhavani (UB) montane swamps of the Nilgiri Mountains (2200 m altitude). In each treatment, 46-50 flowers were emasculated from 15-22 plants at each swamp site. Manipulation experiments demonstrated that the geitonogamy produced, on average, lower fruit set (43%) and seed number ($3.73 \pm 0.18\%$) than the xenogamy treatment (fruit set (92%) and seed number ($7.94 \pm 0.06\%$)). Fruit set (87%) and mean seed number ($7.26 \pm 0.14\%$) in the control set were not significantly different between two sites and no fruit set was recorded from the autogamy. The results suggest that *I. rufescens* is an obligate out-crosser and is partially self-incompatible. The self-incompatibility index (ISI) was calculated as $43/92 = 0.47\%$ (between 0.2 and 1), the ratio of fruit set between the two sites. Each pollinator visit differed significantly from the total number of visits; however diverse insect visit was significantly higher between two sites. It is concluded that outcrossing rate may increase the *I. rufescens* population by greater pollen deposition through manipulation and aid by foraging pollinators.

Key Words: Breeding System; Flower Visitors; Geitonogamy; Swamps; Western Ghats

INTRODUCTION

The reproductive biology and pollination ecology of *Impatiens* have been well studied throughout the tropics (Ornuddff 1969, Rust 1977, Schemske 1978, Heinrich 1979, Schemske 1984, Kato et al. 1991, Anderson 1995, Lu 2000, Sreekala et al. 2008, Ramasubbu et al. 2011). Many hermaphrodite balsam plants reproduce through a range of breeding systems and are pollinated by different floral visitors under natural conditions (Kato et al. 1999, Tian et al. 2004, Sreekala et al. 2008, Ramasubbu et al. 2011). However, most *Impatiens* species throughout the tropics reproduce by cross pollination (Abrahamson and Hershey 1977, Schemske 1978, 1984, Simpson et al. 1985, Schmitt and Ehrhardt 1987, Waller and Knight 1989, Schmitt and Gamble 1990, Twasuda and Yahara 1994, Lu 2000, 2002) and few studies have indicated that lower fruit set is exhibited through self-pollination and

no fruit set is found in autogamous (Tian et al. 2004, Sreekala et al. 2008, Ramasubbu et al. 2011). Nevertheless, more studies from threatened habitats are needed to understand their breeding system and pollination mechanism and to validate earlier studies.

Impatiens is distributed mainly in the Old World tropics, especially Africa, India and other parts of Asia. In India, there are over 200 species and their distribution is highly localized in the Himalaya and the Western Ghats (Chhabra 2006). Of the 92 species found in the Western Ghats, 80 are endemic to the region (Sreekala et al. 2008). More than 35 species of *Impatiens* are known from the upper Nilgiris (Chhabra 2006), a unique montane swamp habitat where very few endemic balsams are found (Mohandass 2008). Several swamps in the region have been converted into grassland and agricultural fields and the prevention of further anthropogenic pressures is vital to conserving endemic

species in this endangered swamp ecosystem (Mohandass 2008, Puyravaud et al. 2012). Some studies on the reproductive biology of several balsam species have been conducted in the Western Ghats; however, the pollination ecology of *I. rufescens* in the Nilgiri Mountains has not previously been examined. Field investigations of *Impatiens* breeding system, reproductive success and pollination under natural conditions are essential for conservation purposes.

Impatiens flowers differ in size, color and shape throughout the tropics and reproductive success depends on various environmental conditions (Tian et al. 2004, Sreekala et al. 2008, Sreekala et al. 2011). *Impatiens* species are pollinated by hummingbirds, bees, butterflies and other insects in subtropical regions of Africa (Grey-Wilson 1980). In temperate zones, some *Impatiens* species are pollinated by bumblebees and hummingbirds (Rust 1977, Heinrich 1979, Kato et al. 1989), whereas in subtropical China, bumble bees are the major pollinators of *Impatiens reptans* (Tian et al. 2004). In the Western Ghats of India, solitary bees, social bees and butterflies have been recorded on several balsam species (Sreekala et al. 2008, Ramasubbu et al. 2011, Sreekala et al. 2011) and they are highly interacting for pollination. Understanding these interactions between plants and their pollinators can provide useful information on reproductive biology, which is vital for conserving rare species in threatened habitats (Kearns et al. 1998). Therefore, this study emphasizes preliminary assessments of reproductive phenology, breeding experiments and pollination systems to understand the pollination ecology of *I. rufescens*.

MATERIALS AND METHODS

The study was carried out in Upper Bhavani reserve forest montane swamp ((11° 14' N and 76° 33' E), of the Nilgiri Mountains, Western Ghats, India. It is located about 60 km away from south of the Ootacamund headquarters in the Nilgiri district. Elevation in the area ranges between 2200 and 2400 m above sea level. The area receives two types of monsoon in each year: Southwest (SW) Monsoon from June through September and Northeast (NE) Monsoon from October to December (Mohandass 2008, Puyravaud et al. 2012). Wind speed reaches up to 120 km h⁻¹ (Dogra and Suprabha 2007) and the mean annual temperature is 14.2° C (Meher-Homji 1987-1988). Swamps form in the hollow depressions of the montane grasslands and shola ridges, but have been

heavily impacted by various human disturbances such as the introduction of invasive species, grazing pressure and agriculture, and are often converted into grassland and degraded land. Some remnant swamps are found in the study area and sustain a varied species composition. However, two endemic Poaceae species, *Andropogon polytychus* and *Eriochrysis rangacharii*, are predominately found.

Impatiens rufescens Benth ex Wight & Arn. is a chasmogamous annual herb species endemic to the Western Ghats of India (Blasco 1970). In 1997, it was listed by the IUCN as “endangered” (Walter and Gillett 1997). *Impatiens rufescens* produces pale or dark purple pink hermaphroditic flowers that hang from auxiliary racemes and offers pollen and nectar as a reward for pollinators.

Population Sampling

From June to November 2007, I randomly established forty 5 × 5 m quadrats in a swamp at each site. The number of flowering individuals in each site was counted to estimate the population size of *I. rufescens* from these quadrats. The total sampled area was 0.1 ha in each swamp. The KR swamp site has a total area of 3.4 ha while the UB swamp site has a total area of 2.5 ha as reported in previous studies (Mohandass 2008; Puyravaud et al. 2012). The distance between these two sites was about 10 km and each population's geographical coordinates and elevation was recorded with a handheld Geographical Positioning System (GPS).

Reproductive Phenology

Reproductive phenology in each of the two swamp populations was observed in 2007 in all forty 5 × 5 m quadrats (0.1 ha) at both sites, and flowering was recorded by visual estimation as follows >1%, 5%, 10%, 25%, 50%, with peak flowering estimated to be >60%. Flower size was measured using a vernier caliper (length × diameter cm). Floral longevity was observed randomly from 30 marked solitary flowers every day, until the perianth fell off.

Breeding System

Breeding system treatments were conducted from July-October 2007. Four treatments were used for the breeding experiments: autogamy, geitonogamy, xenogamy and control. The distance between any set of

manipulation experiments was about 100 m. We used cotton pollination-bags to exclude flower visitors for breeding treatments. The following treatments were carried out:

a). Autogamy: flowers were isolated and bagged without further manipulation.

b). Geitonogamy: flowers were pollinated manually with pollen of different flowers from the same individual.

c). Xenogamy: previously emasculated flowers were hand-pollinated with a mixture of pollen taken from other individuals of the same species.

d). Control (Natural pollination): Unbagged flowers were marked without experimental manipulation (Bawa 1974, Vervoort et al. 2011).

In each treatment, 15-22 plants were selected in each site and 46-50 flowers were manipulated in each treatment. A total of 396 flowers were used about 176 individuals for manipulation experiments in both sites (Table 1).

The degree of self-incompatibility index (ISI) was calculated from the percentage of geitonogamy fruit set divided by the percentage of xenogamy fruit set. Species with a ratio between 0.2 and 1 are considered partially self-incompatible, those with a ratio between < 0.2 and 0 are completely self-incompatible and those with a ratio > 1 are self-compatible (Ruiz-Zapata and Arroyo 1978, Subasi and Guvensen 2011).

Floral Visitors

Pollinators were observed and recorded for 12 h in each swamp site during the peak flowering period. A pollinator's visitation rate was recorded in twenty minutes for every one hour intervals between 7:00 and 12:00 h within the 4-m² quadrats of each swamp (Larson and Barrett 1999). Foraging pollinators were recorded and identified.

Statistical Analysis

Population density was summed-up and compared between the two sites using a chi-square test. The percentage of fruit and seed set was calculated from the total number of flowers in each of the different breeding treatments. From the result of different treatments, data on fruit-set and seed set were compared statistically

using chi-square and independent sample t-tests. ANOVA was used to determine variation in the total number of visits and I calculated average visits for 12 h observation period among different pollinators.

RESULTS

Population Density

A total of 1979 individuals were recorded in the study areas, with 1091 individuals ^{-0.1ha} in the KR site (55 %), and 888 individuals ^{-0.1ha} in the UB site (45 %). The mean population density for all sites combined was 989.5 ^{-0.1ha}, however population density differed significantly between the two sites ($\chi^2 = 19.19$, $df=1$, $P < 0.0001$).

Reproductive Phenology

The flowering period lasted from the beginning of the south-west monsoon (early June) until the end of the northeast monsoon (late November) in both sites (Figure 1). The average life-span of each individual flower was 5.3 days ($n = 30$, $SD = 0.3$) and the perianth fall-off occurred after 7.5 days ($n = 30$, $SD = 3$). Flower size ranged from 1.7 to 2.3 cm and average flower size was 1.94 ± 0.04 cm ($n=30$). The fruiting phase occurred after perianth fall-off.

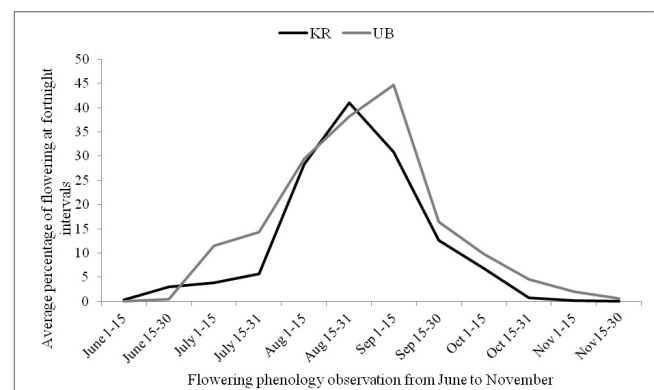


Figure 1. Proportion of flowering phenology between the two study sites during the year 2007.

Breeding System

In the manipulation treatments, there was no fruit set in the autogamy treatment for the two sites. The fruit set in

the geitonogamy and xenogamy was 41% (n = 46) and 90% (n = 50) in KR, and 44% (n=50) and 94% (n = 50) in UB, respectively. There was a significant difference in fruit set between geitonogamy and xenogamy treatments between the two sites ($\chi^2=23.67$, $df = 1$, $P < 0.0001$ in KR, and $\chi^2 = 26.93$, $df=1$, $P < 0.0001$ in UB). The mean seed number in the geitonogamy and xenogamy was $3.6 \pm 1.65\%$ (n=19) and $7.9 \pm 0.99\%$ (n=45) in KR and $3.86 \pm 1.43\%$ (n=22) and 7.98 ± 1.50 (n=47) in UB, respectively. It indicates a significant difference in mean seed number between the geitonogamy and xenogamy in two sites (t -test = 12.74, $df = 62$, $P < 0.0001$ in KR, and t -test = 10.79, $df = 67$, $P < 0.0001$ in UB, independent samples t -test). Similarly, fruit set and mean seed number between the geitonogamy and control treatments was significantly different (Table 1). These results suggest that fruit set and seed number was significantly higher in xenogamy and control treatments than in geitonogamy and indicate that transfer of more pollen deposition stimulates higher fruit set with the aid of cross pollination and foraging pollinators. Moreover, the pollination experiments resulted to an index of self-incompatibility (ISI) between 0.2 and 1 and suggests that *I. rufescens* is an obligate outcrossing species and partially self-incompatible.

Flower Visitors

Four groups of pollinators were observed on this species namely, *Apis cerana*, carpenter bees (*Xylocopa* sp.), flies, and diverse insects (including *Trigona* - a small bee, and other tiny insects). The mean pollinator visits from each of the four groups varied significantly (ANOVA $F=68.14$, $df=7$, $p<0.001$). Visitation generally began soon after sunrise and typically declined in the afternoon. In the 4m² quadrats, *Apis cerana* made up 12% (N=68), carpenter bees 19% (N=107). The majority

of floral visitors included diverse insects 38% (N = 210) and flies 31% (N = 174) in both sites (Figure 2). *Apis cerana* and carpenter bee's floral visits were not significantly different between the two sites, however visits by diverse insects and flies were differed significantly (Table 2).

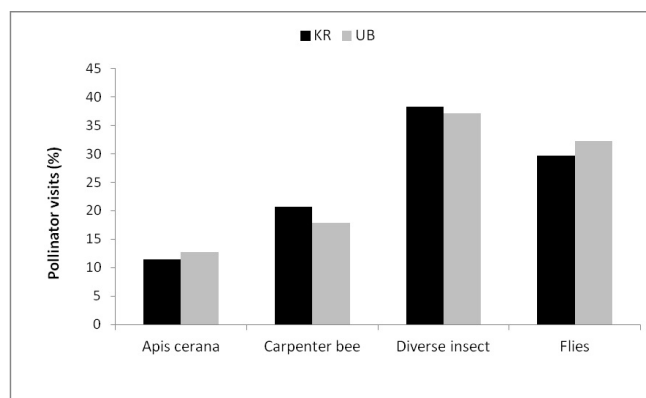


Figure 2. Observation data for pollinator's visitation rate of *I. rufescens* over 12 h periods at two study sites

DISCUSSION

Since the autogamy treatment did not produce any fruit set in both study sites, this study indicates that *I. rufescens* is an obligate outcrosser and a partially self-incompatible species that strictly depends on pollinators to set fruits. Several pollination studies have shown that other members of the family Balsamineae are self-incompatible (Kato et al. 1991, Sreekala et al. 2008, Ramasubbu et al. 2011). Morphologically, the arrangement of stamens, pistil and spur are markedly adapted for cross pollination in *Impatiens* (Bhaskar and Razi 1974) and hence most *Impatiens* species reproduce by cross

Table 1. Breeding experimental treatments of *I. rufescens*, showing the number of flowers treated, and resulting number of fruit set (%) and number of seed set (mean \pm SD) between the two sites

Variables	Autogamy		Geitonogamy		Xenogamy		Control	
	KR	UB	KR	UB	KR	UB	KR	UB
No. of flowers	50	50	46	50	50	50	50	50
No. of fruit set	0	0	19	22	45	47	43	46
Fruit set (%)	0	0	41	44	90	94	86	88
No. of seeds (mean \pm S.D)	0	0	3.6 ± 1.65	3.86 ± 1.43	7.9 ± 0.99	7.98 ± 1.50	7.16 ± 2.00	7.36 ± 1.76

Table 2. Observation data for pollinator's visitation rate of *I. rufescens* over 12 h periods at two study sites

Site	Total visits	Flowers visited per visit (Mean \pm SD)			
		<i>Apis cerana</i>	Carpenter bee	Diverse insects	Flies
KR	246	1.87 \pm 0.83	3.4 \pm 1.92	6.24 \pm 1.53	4.56 \pm 2.13
UB	313	2 \pm 0.79	2.84 \pm 2.35	5.8 \pm 1.51	5.05 \pm 1.05
Comparison		$T = 0.65$	$T = -1.34$	$T = -2.09$	$T = 2.01$
Test		$df = 66$	$df = 105$	$df = 208$	$df = 172$
		$P = 0.52$	$P = 0.18$	$P = 0.04$	$P = 0.05$

pollination (Schmitt and Gamble 1990, Lu 2000, 2002). However, the study species is well maintained in the natural environment, and is not pollen limited for fruit and seed production and thus reproductive success leads to a hermaphroditic partially self-incompatible system.

The low average fruit set in the geitonogamy treatment (43%) compared to the xenogamy treatment (92%) indicates shorter distance of pollen transfer limited fruit production and plant fitness and thus showed significant variation in reproductive success among manipulations treatments. It suggests that *I. rufescens* received pollen from far distance that ensures stronger ability of fertilization. However, compared to open pollination, xenogamy produced a slightly higher fruit set indicating that outcrossing manipulation is more effective than natural pollination. Lloyd (1992) and Eckert (2000) suggested that geitonogamy is not effective for hermaphrodite species, probably resulting in severe pollen scarcity and seed discounting and providing little reproductive assurance. In addition, geitonogamy leads to self-fertilization and lower seed number, thus less fruit set may cause a reduced female reproductive success. The study also demonstrates that *I. rufescens* exhibits protandry in the first stage of development that lasts for 2 days. Subsequently, when the protogyny stage receives more pollen aided by floral visitors that enhances female fertility, it can change from a male to a female phase.

Interestingly, *Impatiens* flowers attract different pollinators in tropical, subtropical, and other temperate zones (Rust 1977, Heinrich 1979, Kato et al. 1989, Tain et al. 2004, Sreekala et al. 2008, Ramasubbu et al. 2011, Sreekala et al. 2011). The present study indicates that a diverse range of insects, including small bees and flies, contribute more to pollination and outcrossing pollen deposition than other pollinators (Table 2). Solitary

carpenter bees (*Xylocopa spp.*) visit *I. rufescens* more frequently than *A. cerana*, but carpenter bees have rarely been observed to pollinate *Impatiens* species. *Apis cerana* is an occasional visitor to *I. rufescens* flowers, attracted to the dark purple pink flowers and displays on sunny days. Therefore, different morphological structures of *Impatiens* flowers attract different types of pollinators. However, it sometimes lacks pollinators due to complex morphological features. For instance, a recent study on *Impatiens chlorosepala* in China revealed the occurrence of long spurs (size: mean length 52.89 mm, N=32 and mean diameter 35.52 mm, N=32) with a nectar standing crop at the end of the spur. *I. chlorosepala* seems to lack common pollinators, but nectar robbers are found to be common because the spurs have many tip holes. Moreover, it sets fruit through autonomous autogamy (Mohandass, unpublished data).

In the upper Nilgiris, 82% of *Impatiens* species flower between June and December, 10% between April and June, and 8% between January and March (Chhabra 2006). Similar observations were made in the Idukki District of Western Ghats where about 62% of *Impatiens* species in the Western Ghats flower from July-December, 16% from April-June and 15% from January-March (Sreekala et al. 2011). The longer flowering period in this study might be a result of high altitude, cloudy weather, prolonged rain, and reduced photoperiods under natural conditions within the study area.

In conclusion, the balsam population is increasing due to outcrossing pollination systems aided by foraging pollinators and adaptation to a specific habitat, as well as a micro environmental niche and soil moisture content. However, *I. rufescens* populations may be at risk due to habitat loss from disturbances such as grazing pressure (Puyravaud et al. 2003, Mohandass 2008, Puyravaud et al. 2012), which may lead to species extinction. It is

therefore emphasized that protection of montane swamps should be a high priority for the conservation and management of this endemic balsam.

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