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Pollination System and ex Situ Fruit Set in *Ceropegia juncea* Wight (Apocynaceae)-an Endemic Species of India

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Abstract: Pollination in the species of *Ceropegia juncea* Wight (Apocynaceae: Ceropegiae) in Southern India is discussed based on observation from *ex situ* grown plants. The study confirmed pollinators are small, mostly female Dipteran flies, ca 3 mm long, which carry pollinaria on the proboscis. The most important factors for an insect to effect pollination appear to be the appropriate overall size, mouth parts and adjacent pads on which a pollinarium can attach. The complex and diverse floral morphology of the tubular corolla and their disposition as well as other commonly occurring features namely, vibratile corolla lobes and hairs, specialized hairs on corona, interior sculpturing, sliding zone and differential lighting within the flower are important mechanisms for attracting insect pollinators. Stigmatic receptivity performs at anthesis and remains so far 24 hr. Flowers show pronounced protogyny. Manual pollinations showed that the species permits geitonogamous pollination. The percentage of fruit set in manually pollinated flowers is higher than that resulting from open pollination, confirming that pollination is a limitation for fruit set in the *ex situ*-grown population. Nevertheless, fruit and seed set are sufficiently high for *ex situ* conservation purposes.

Key words: *Ceroepgia juncea* · Medicinal plant · Dipteran fly · Geitonogamy · Pollination biology

INTRODUCTION

Ceropegia L. is a large genus of about 200 species in the family Apocynaceae (Ceropegiae) and occurs in the old world tropics and subtropics. *Ceroepgia juncea* Wight, a twining leafless tuberous succulent herb is distributed in drier parts of Peninsular Inida and is well known medicinal plant since the Vedic period. The alcoholic extracts of the plant is reported to possess antipyretic, analgesic, antiulcer, hepatoprotective, tranquilizing and hypotensive activities in experimental animals [1]. Ceropegin, a novel furopyridine alkaloid isolated from this species and it has shown significant analgesic effect against acetic acid writhing mice [2]. Due to habitat encroachment and overexploitation for medicinal purposes in local medicine, the species has become rare in its natural habitat.

MATERIALS AND METHODS

Wiry succulent stems and seeds were collected from natural habitats of Dindigul district of Tamilnadu and Anantapur district of Andhra Pradesh during the year 2006-2007, saplings and cuttings have been established at the Botanical garden of Sri Krishnadevaraya University, Anantapur andhra Pradesh. They have grown well and are flowering regularly. Each plant produces a normal rate of flowering ($\geq$15) with some morphological variations.

Breeding system was studied by carrying out manual pollinations and recording fruit set. The flowers were bagged and tagged for respective studies before anthesis. Pollinators of bagged flowers were carried out 0-24 hours after pollinia maturation by cutting a 0.5 sq cm window in the basal chamber of corolla tube and pick the pollinaria taken on the tip of a needle onto the stigma. Pollinated flowers were bagged and marked. Manual autogamic self-pollinations could not be carried out as the stigmas had dried up and lost their receptivity by the time, the pollinaria of the same flower opened. Geitonogamous pollination (pollinaria from another flower of the same plant) and xenogamous pollination (pollinaria from another plant) were carried out manually. Thirty five flowers were bagged and left without manual pollination as additional test for autogamy. Fifty opened flowers were tagged to assess fruit maturity was also monitored until dehiscence.

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RESULTS AND DISCUSSION

Flowers are shortly pedicelled, bisexual, zygomorphic with superior ovary and are produced in axillary cymes (Fig. 1a). The corolla tube is 5 cm long, 1.5 cm wide with inflated base, then funnel-shaped, lobes about as long as the tube, connate at the tip (Fig. 1b). The outer corona-lobes are deeply bifid, ciliate, inner corona linear-spathulate, hooked at the tip. The variations observed from the flowers of Tamilnadu specimens, show the long purple cilia on margins of lobes and short white cilia on inner folding lines of lobes (Fig. 1c). Both the outer and basal part of inner corona covered with dense purple cilia, whereas in Andhra Pradesh specimen flowers without cilia on corolla lobes and very sparse cilia on the outer corona lobes. The purple blotches on the outer surface of corolla tube is markedly varied from the both specimens. *C. juncea* floral structures are with significant roles in pollination namely, the pollinarium, the gynostegium and the corona. The pollinarium is composed of two pollinia, each containing all of the microspores of a single anther locule embedded in a hard matrix and a translator apparatus (corpusculum and caudicle) (Fig. 1d). The translators are specialized transportation devices to help in attaching the pollinia to the pollinator. The gynostegium is composed of the post-genitally united stamens and carpels of the flower. The corona is composed of one or more whorls of structures attached to, or located between the corolla and the androecium. Among the functions of corona are providing optical cues to pollinators for pollinia removal and insertion by mechanically positioning the proboscis.

In *C. juncea* small two-winged Dipteran flies carried pollinaria on their proboscis (Fig. 1). Pollination was effected when a pollinarium got detached from the proboscis and lodged at the base of a guide rail and eventually germinated. The flies were trapped in the corolla tube for about a day until the downward pointing stiff hairs became limp and allowed them to escape and in the process, they sometimes carried pollinaria and took them to another flower. The mechanisms of insect attraction and retention, mainly colour and light effects, scent, specialized hairs, sliding zone, nectar and the complex corolla morphology were identified. Apart from

Fig. 1: *Ceropegia juncea*: a, Flowering plant under *ex situ* b, Longitudinal section of the flowers; c, Corona top view; d, Pollinarium; e, Lateral lobe of pollinia; f, Pollinarium attachment on the proboscis of Dipteran fly

the complex morphology, *Ceropegia* flowers are most striking due to their very colourful patterns (Fig. 1b). Colour is an important floral attribute and functions as a long distance signal especially to actively flying diurnal insects such as the Diptera. Dipterans, that are mostly tiny, two winged flies in the size ranges of *Drosophila*, are the only known pollinators of *Ceropegia* [3]. The presence of pollinaria on mouth parts alone and not on any other part of the body of the flies shows the importance of suitable mouth parts in *Ceropegia* pollinators [4]. Suitable mouth parts constitute an extendible proboscis for probing that has a surface on which the pollinaria parts can firmly attach (Fig. 1f). The extendible proboscis can probe in the often constrict and complex area of the corona to reach the pollinaria and nectaries.

C. juncea is usually growing in the sheltered places at the bases of thickets, among the thorny bushes and scrub edges. This is low climbing species; flower is a few meter above the ground. The vegetative parts and the flowers are usually well camouflaged in their habitat and as a result, they are very difficult to spot in the field even when in flowering. The various floral adaptations such as the vibratile parts, colour and scent may assist the pollinators to find the flowers. *C. juncea* pollinators are small; it may be assumed that their flight distances are limited. The small flies would probably not be able to withstand winds and adverse weather conditions when flying even for short distances. The hidden and shelter habitat of *C. juncea* are calm areas when the flies can go about their activities without much disturbance from winds and other detractors such as adverse weather.

C. juncea inflorescence produced 3-5 flowers on axillary cyme and they open simultaneously. These flowers are mostly produced while sheltered within the associated leafy species. The individual flowers are frequently oriented by the curvature of the corolla tube. The bases of the corolla tubes are somewhat whitish-translucent, such a upside-down disposition provides a strong light signal that attracts the Dipteran flies already within the flower towards the base of the corolla tube. The pollinators are strongly attracted to the coronal area and the light window effect provides a false signal for an exit route in the region of the base of the corona. A fly bringing foreign pollinaria or picking pollinaria from a visited flower are thus enhanced.

The corolla of *C. juncea* is the most colourful, ornamented with purple blotches on the yellowish-green ground colour. In typical flower, a long, closed, usually basally inflated corolla lobe raises high above the corona. This tube terminates in five lobes that are often of considerable length pocket-like aperture at the base of the lobes provide access to pollinating insects to the anterior of the tube and down to the corona. Downward pointing ciliate hairs are frequently present inside the tube and its inflated bulbous base, often to retain pollinating insects in the neighborhood of the sexual organs of the flower.

In *C. juncea*, corona is a biseriate structure comprising five outer and five inner lobes attached to the central gynostegium in the middle. The corona is deep purplish coloured or in inner corona mixed with yellow colour and adorned by stiff hairs that also purple coloured. The most important functions of the corona in pollination is probably to provide optical cues to flies and guide them into the appropriate positions for pollinia removal and insertion by mechanically positioning the proboscis. The outer corona lobes are cupular and frequently divided and play an important role of restricting the insect pollinator next to the pollinaria and nectaries. The inner lobes are linear, erect, free from the base to the apex and variously coloured.

The guide rail of *C. juncea* is light creamy or ivory in colour and its colour do not lose when the flowers are pressed or stored in liquid preservatives. The guide rail is so named because they guide the insect proboscis and legs towards the corpusculum. The bases of the guide rails shortly project above the nectar cavities.

C. juncea pollinaria are generally ovoid and sub-erectly oriented on the stylar head (Fig. 1c). Pollinia are usually yellowish and have distinct cell ornamentation (Fig. 1e). They are quite conspicuous and mostly well exposed and can therefore easily attract pollinating insects. The pellucid margin, which is the germination mouth for the pollen, occurs on the sub-lateral region of the inner margin. The carpusculum, which is the central part of the pollinarium connected to the pollinia by a pair of caudicles. The corpusculum and caudicle constitute the translator apparatus. A pollinating fly picks the whole pollinarium from the stylar head but will usually lose it in two stages by having one pollinarium break off first followed by the second either on the same flower or on a different flower.

The present study experimented by dissecting the flowers and placing pollinia at the entrance to the nectar cavities at the base of the guide rails and they germinated after about 12-24 h. The pollen tubes grew into the nectar cavity beneath the guide rail to the carpels regardless of the position in which the germination mouth was facing. The maturation of pollinaria observed only when the opening of flower usually at morning 7-8 am. The mature pollinaria have loosened the lobes and attached only the flap-like membranes behind the corpuscle. The flies were

Table 1: Fruit set of manual geitononganous and xenogamous pollination of *C. juncea* under *ex situ* condition

Hours after	Geitonogamous No. of flowers	pollination % of fruit set	Xenogamous No. of flowers	pollination anthesis % of fruit set
0	20	22.4	15	32.5
01	20	89.2	20	92.3
02	20	93.5	20	96.5
03	20	62.1	18	85.4
04	20	59.2	20	61.2
05	20	23.8	20	50.1
10	20	20.6	19	40.5
15	20	9.3	20	30.4
20	20	6.4	22	25.7
25	20	2.1	20	12.6
30	20	1.0	15	10.1
35	20	0.0	18	2.6
40	20	0.0	15	0.0
45	20	0.0	15	0.0

Figure 1. *Ceropegia juncea*: *a*, Flowering plant under *ex situ b*, Longitudinal section of the flowers; *c*, Corona top view; *d*, Pollinarium; *e*, Lateral lobe of pollinia; *f*, Pollinarium attachment on the proboscis of Dipteran fly

counted in each flower from the 20 different individual plants. The average number of flies was observed in each flower and they are carrying at least one pollinaria by their proboscis.

Bagged flowers without manual pollination did not set fruits, confirming the absence of autogamy in the species. Out of the 50 flowers exposed to open pollination, only 13 fruits set. However, both geitonogamous and xenogamous pollinations resulted in a significantly higher number of fruit set (Table 1). In both types of pollination, the percentage of fruit set was high when the flowers were pollinated first and second hour of anthesis. The fruit set gradually decreased until 48 h, after which no fruit set was observed. This was in conformity with the data on stigma receptivity. There were not more variations in the size of the fruits and number of seeds per fruit from both pollinations.

Most of the species of *Ceropegia* so far are reported to be pollinated by Dipteran flies including Milichidae, Chloropidae and Ceratopogonidae [5,6]. Some species of *Ceropegia* reported that their flowers provide for larval development and breeding site respectively to the pollinators. In *C. juncea* flowers also have observed white cloured larvae and hatchlings inside the inflated region of corolla tube.

The present findings on *C. juncea* indicate that have well developed fly-trapping mechanisms and that pronounced protogyny precludes autogamy. However, the prevailing breeding system permits geitonogamy. Most of the *Ceropegia* species such as *C. cumingiana* [7], *C. nilotica* [8] and *C. elegans* [9] which are self-incompatible. The well marked difference in the percentage of fruit set resulting from open pollination and manual pollination in *C. juncea* clearly shows that pollination is limitation factor for fruit set under the prevailing conditions. The results are also in agreement with the observations that insects were recorded 95.8% of the flowers during the stigma-receptive phase. However, the number of follicles (>10) and seeds (>200) per follicle produced by *C. juncea* plants even under ex situ conditions is quite adequate and offers a distinct advantage for conservational efforts.

REFERENCES

1. Pullaiah, T., 2006. *Encyclopaedia of world medicinal plants*, I vol, Regency publication, New Delhi.
2. Sukumar, E., G.R. Hamsaveni and R.R. Bhima, 1995. Pharmacological action of ceropegin, a novel pyridine alkaloid from *Ceropegia juncea*, *Fitoterapia,* 66: 403-406.
3. Ollerton, J. and S. Liede, 1997. Pollination systems in the Asclepiadaceae: a survey and preliminary analysis, Biol. J. Linn. Soc., 62: 593-610.
4. Chadurvedi, S.K., 1993. Significance of light windows in the pollination of some Indian Ceropegias (Asclepiadaceae), Cat. Succ. J. (US)., 65: 148-151.
5. Masinde, P.S., 2004. Trap-flower fly pollination in East African *Ceropegia* L. (Apocynaceae), Intl. J. Trop. Insect Sci., 24: 55-72.
6. Ollerton, J. and P. Forster, 1995. Diptera associated with flowers of *Ceropegia cumingiana* in Australia, *Asklepias*, 66: 21-22.
7. Fishbein, M., 2001. Evolutionary innovation and diversification in the flowers of Asclepiadaceae, Ann. Missouri Bot. Gard., 88: 603-623.
8. Ollerton, J., 1999. Fly trapping in *Ceropegia* flowers-Evidence of ant predation of pollinators, *Asklepias*, 77: 31-32.
9. Kunth, P., 1909. *Handbook of flower pollination*, Vol. III. Translated from the original in German by J.R. Ainsworth Davis, Clarendon Press, Oxford, pp: 644.