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Reproductive Biology of the *Hamelia patens* Jacq. (Rubiaceae) in Northern India

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ABSTRACT

A study of floral biology and the breeding system of *Hamelia patens* Jacq. (Rubiaceae) was carried out in northern India from March, 2005 to February, 2007. The data obtained was compared with those obtained from the original area of distribution of the species. This species is an ornamental shrub cultivated in the gardens for its beautiful orange-red bunches of flowers and copper green leaves. The tubular flowers are yellowish orange or orange-red, 3.05±0.12 cm long and last for three days. They are protrandous and the pollen is available 12-14 h before the stigma becomes receptive. The beginning of anthesis takes place between 0230-0300 h and the opening of the bifid stigma occur in the afternoon between 1430-1700 h. The nectar is secreted during both the male and female phases, with higher concentrations of sucrose. The floral biology is related to environmental factors, particularly temperature. The quantity of nectar and pollen fertility declines with the rise in temperature and in summers when temperature ranges between 37° C to 45° C, nectar was more or less absent and only 2-3% pollen were fertile. The flower visitors included honeybees, butterflies, wasps, house flies, ants and sunbirds. Ants and house flies are robbers whereas; all the others act as pollinators. Squirrels were frequently seen, but they just eat away the base of the flower full of nectar. The plant is self-compatible and facultative xenogamous. The natural fruit-set is only 7% but seed-set rate is slightly higher. Thus, this species growing in its natural habitat in Argentina and in its cultivated form in India exhibits some differences in pollen fertility, stigma receptivity, nectar quantity and quality and behaviour of flower visitors and absence of hummingbirds from India. These are responsible for the difference in the reproductive success of this species at two places. It seems that this species is under the process of acclimatization in North India.

Keywords : *Hamelia patens*, nectar, pollen fertility, fruit-set, pollinators

INTRODUCTION

The family Rubiaceae has 10,700 species consisting of trees, shrubs and herbs (Robbrecht 1988). The family is cosmopolitan in distribution but distributed mainly in tropical regions (Bacigalupo 1993). The three subfamilies (Bremer 1996) show three main reproductive mechanisms: heterostyly is common in Rubioideae, secondary pollen presentation on the style in Ixoroideae, whereas Cinchonoideae presents both mechanisms and anemophily is restricted to more advances tribes of Rubioideae (Robbrecht 1988). All these mechanisms are relatively constant within the tribes (Robbrecht 1988). Pollination syndrome and breeding system in various members of the family Rubiaceae has received considerable attention in new world (Kay 1978, Thomas *et al.* 1986, Nilsson *et al.* 1990, Zurovchak 1997, Lara & Ornelas 2001 and Lasso & Naranjo 2003). However, *Hamelia patens* in India shows considerable variation in floral morphology, pollination and fruit set in different seasons. Keeping this in view, present investigation has been undertaken to study floral morphology, pollination syndrome, breeding behaviour and pollen-pistil interaction in cultivated populations

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of *Hamelia patens* at Agra, in order to compare these data of cultivated plants with those obtained within its area of natural distribution.

MATERIAL & METHODS

A. FLORAL MORPHOLOGY — A total of 20 flowers each from twenty individuals of *Hamelia patens* plants growing in different gardens namely, Moti Lal Nehru Park, Paliwal Park, and Sur Udyan, School of Life Sciences, Khandari campus of the University, Agra, India (26° 44′ and 77° 55′N and 78° 32′E) were monitored from anthesis to flower abscission during a period of three years (March, 2005-February, 2007). Pollen viability was tested in 20 flowers each (three anthers per bud) in all the individuals, using Alexander's (1980) staining procedure, *in vitro* hanging drop culture method (Brewbaker and Kwack 1963) and *in vivo* aniline blue fluorescence microscopic method (Heslop-Harrison & Heslop-Harrison,1970). Stigma receptivity in both pre- and post-anthesis stages was determined through the peroxidase activity technique (Kearns & Inouye 1993) between 05.30 and 20.00 h at two hours intervals, (n= 20, from ten individuals). Pollen load on stigmatic surface was studied by mounting them in basic fuchsin gel and examined under the microscope (Kearns & Inouye 1993). Floral morphology data were collected from fresh flowers collected in both male and female phases. Length and diameter of the corolla, stamen filament and style were measured with caliper (error: 0.01 mm) on a sample of fresh flowers (days 1 and 2, n= 50) from *ca.* 20 individuals. Pollen/ovule ratio (Cruden 1977) was calculated by counting pollen grains (stained by acetocarmine) from a half of a thecae of anthers from ten flowers, and all the ovules from ten pistils; in both cases one flower was used per plant, and the observations were made under a microscope.

B. REPRODUCTIVE BIOLOGY — Hand pollination treatments were done using flowers at the time of stigma receptivity from 22 individuals, between 1400 h to 1630 h. The un-emasculated flowers (except for checking apomixes) were bagged during pre-anthesis stage with muslin cloth bags and the flowers received more than one kind of treatment. Pollen was transferred by bringing the dehisced anthers in contact with the receptive stigma with the help of a forceps holding the anther filament alone. Self-pollinations (n=60) and cross-pollinations (n=75) using non-neighbour plants were performed, and differences on fruit set between these pollinations were determined using a Chi-square test. Emasculations in pre-anthesis floral buds were done to test for the presence of apomixes (n=52). Open flowers were tagged to test fruit set under natural conditions (n=69).

Pollen tube growth resulting from both self- and cross-pollinations were investigated using fluorescence technique (Shivanna & Rangaswami 1992) in the pistils fixed 24, 48 and 72 h after hand pollination. Fruits from the plants under observation were counted to estimate the individual fruit set.

C. NECTAR — Nectar volume and sugar concentration were measures on 50 bagged flowers from 10 individuals, using calibrated glass capillaries and hand refractrometer (Dafni 1992). The values on the refractometer were considered as sucrose equivalents (see Inouye *et al.* 1980). These data were collected with one hour intervals approximately from buds and at the beginning of anthesis till weathering of the corolla from the same flowers (n=10). The nectar of another set of bagged flowers (n=10) was collected at same time on the second day, representing accumulated values. Student t-test was used to compare the values of nectar volume and concentration. Tests of amino acids, proteins, lipids, phenols, alkaloids, and reducing acids were performed after Baker & baker (1975). Sugars were separted using paper chromatographic method after Grant & Beggs (1989) using Whatman number 1 filter paper. Three descending solvent systems used were: a. ethyl acetate-pyridine-water (8:2:1, v/v); b. n-butanol-glacial acetic acid-water (3:1:1, v/v); c. 1-propanol-ethyl acetate-water (7:1:2, v/v). Dried chromatograms were treated with p-aminobensoic acid to detect sugars.

D. FLORAL VISITORS — Floral visitors and their behaviour were recorded from 05.30 h until 21.30 h, for a total of 48 h of focal observation. The entire period was sampled with the same effort, but on different days. Pollinators were determined based on frequency of visits and the visiting behaviour i.e. legitimate visits on the basis of contact with the reproductive parts (anthers and stigma).

RESULTS & DISCUSSION

A. FLORAL MORPHOLOGY — *Hamelia patens* flowers throughout the year. However, flowering phenology is quite variable and under the control of environmental conditions. The inflorescence is polychasial cyme (Fig. 1a) and during September-February with temperature ranging between 6°C and 30°C, the number of cyme/plant is 97±21 and each cyme consists of 45±3.5 floral buds (n=36-54), of which 5±2 are open flowers and 40±5.5 are buds of different size (Fig. 1a). The open flowers measure 3.05±0.12 cm with 0.03±0.012cm diameter of the corolla. Each flower lasts for 2-3 days before the ovaries either start swelling into fruits or abscise. The flowers are orange red in colour, chasmogamous, sub-sessile, ebracteate,

Fig. 1a-f — *Hamelia patens* with different vistors. **a.** Flowers with butter fly. **b.** Flowers with honey bee. **c.** Flowers wi female greenish-yellow sun bird *(Nectarinia asiatica)*. **d.** Squirrel eating flowers. **e.** Flowers with male (purplish black) su bird. **f.** *In vivo* pollen germination on the stigmatic surface (pollen tubes-PT).

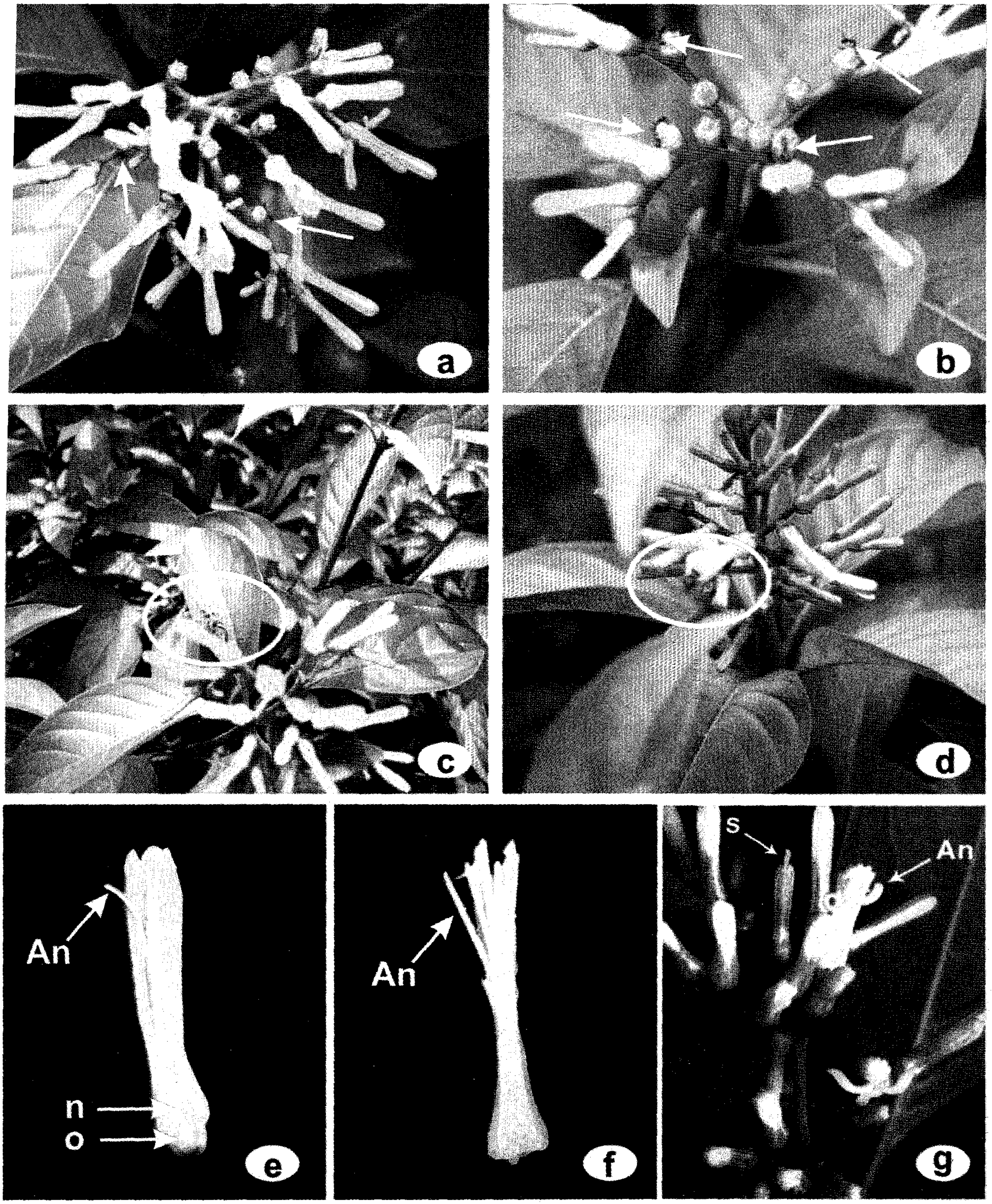


Fig. 2a-g — Floral morphology of *Hamelia patens*. **a.** Orange red flowers with ants (arrow). **b.** Flowers and young fruits with ants (arrow). **c.** Flowers with a spider. **d.** Flower with a house fly. **e.** LS mature flower showing ovary (o), nectary (n) and dehisced anthers (An). **f.** Mature flower after removing petals showing dehisced anthers (An). **g.** Flowers showing protruding stigma (S) and protruding anthers (An).

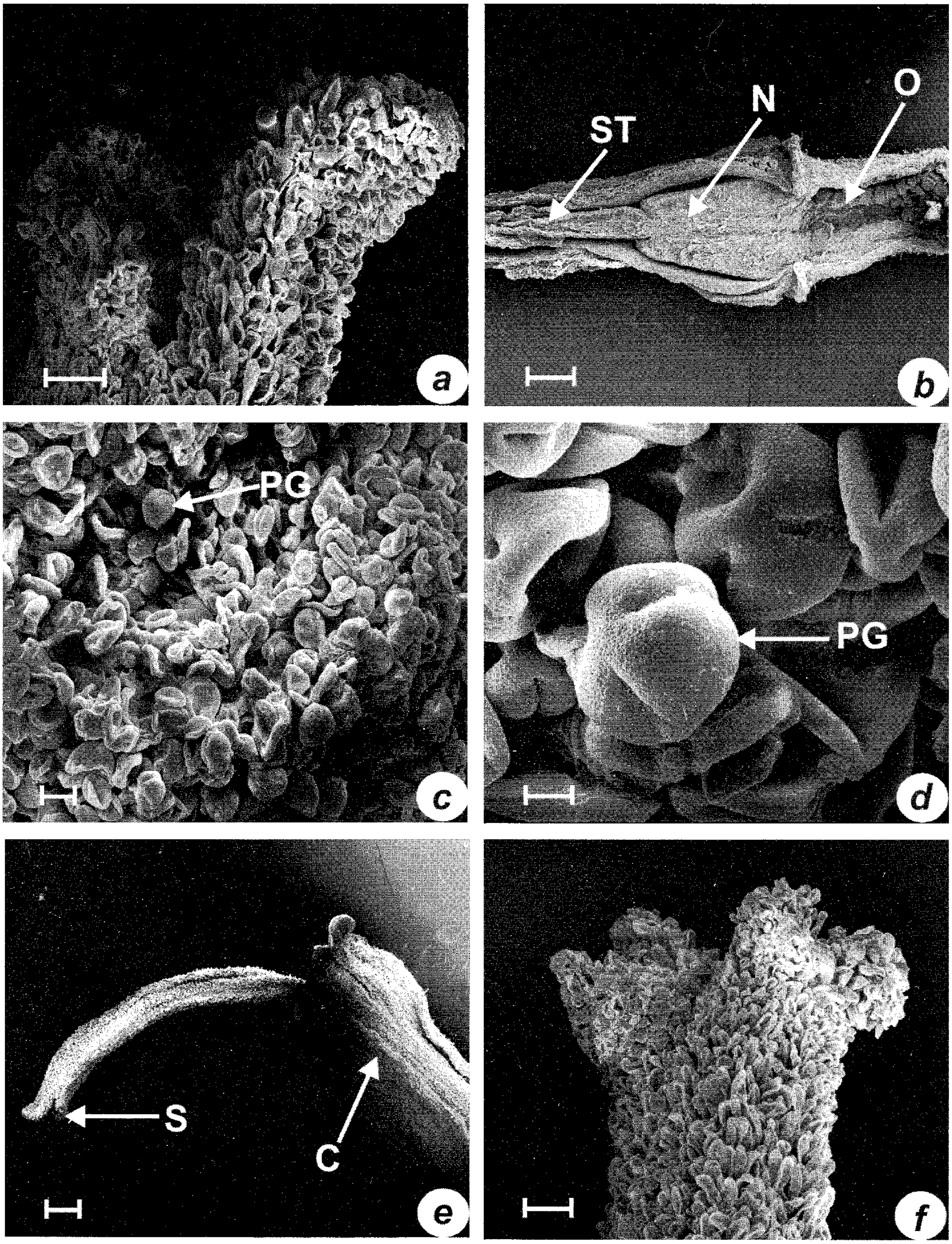
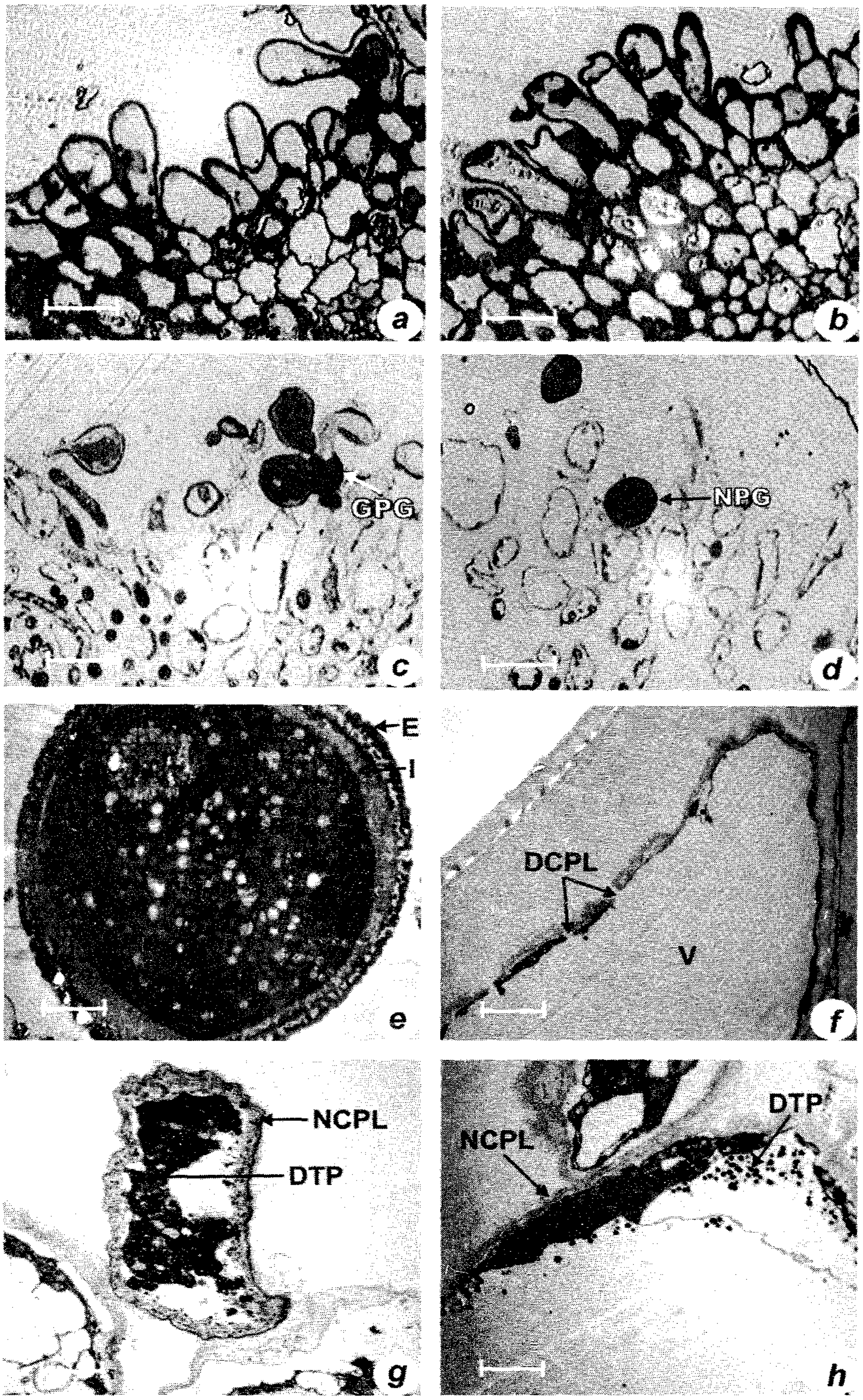


Fig. 3a-f — Scanning electron microphotographs of pistil and pollen grains. **a.** Bifid and papillate stigma. Bar 30 **b.** L. S. of pistil showing a symmetric nectary (N) disc surrounding the basal portion of the style (ST) and above the c (O) with ovules. Bar 200μm. **c.** Pollen grains (PG) on the papillate stigmatic surface. Bar 10μm. **d.** Magnified view of c 2 showing tricolpate pollen grains (PG) with reticulate exine. Bar 3μm. **e.** Stigma (S) protruding from the corolla (C). 300μm. **f.** Tetrafid stigma. Bar 30μm.



ig. 4a-h. Light microscopic (a-d) and Transmission electron microscopic (e-h) photographs of pollen grain and stigmatic ıpillae. **a.** Vertical section of stigma in open flower showing thin walled, medium sized and loosely arranged papillae. Bar 0μm. **b.** Vertical section of protruding stigma showing thick walled, elongated and compactly arranged papillae. Bar 10μm. Open flower stigma with germinated pollen grain (GPG). Bar 10μm. **d.** Protruding stigma with non-germinated pollen grain ΝPG). Bar 10μm. **e.** Mature pollen grain full of reserve food showing well developed exine (E) and intine (I). Bar 5μm. Highly vacuolated (V) papillae of open flower showing disrupted cuticle pellicle layer (DCPL). **g & h.** Protruding stigma ith thick walled non-disrupted cuticle pellicle layer (NCPL). Bar 5μm.

hermaphrodite, actinomorphic, epigynous and complete. The calyx is gamosepalous with five sepals, adnate to the ovary wall and arranged in valvate aestivation. The corolla is gamopetalous with five petals, tubular and arranged in twisted aestivation. They are reddish orange in bud condition, but their upper half turns yellowish orange at the time of anthesis and turn gradually to orange by the same evening (Fig. 2a). The next morning, they are reddish orange and by the evening of the third day, they begin to wither. On the fourth day, the pollinated flowers drop off their corolla and stamens, while un-pollinated ones begin to abscise. The stamens are five, polyandrous, alternate to the petals and their filaments are fused at the basal part of the corolla tube. The anthers are basifixed, long, bi-celled, introse and borne on short filaments. The style is long with a bifid and papillate stigma (Fig. 3a, c). A symmetric nectary disc is located surrounding the basal portion of the style (Figs. 2e, 3b). Similar floral morphology in *H. patens* plants growing at Tucuman, Argentina has been recorded by Galetto (1998). He has observed several stomata distributed all over the nectary surface. All the nectaries are supplied by xylem and phloem bundles. The secrectory parenchyma is differentially stained and composed of cells with large nuclei and vacuoles (Galetto 1998).

B. REPRODUCTIVE BIOLOGY — During the period between September-February, the flowers start to open around 0300 h and are fully open at 0330 h (Fig. 2a). In the months of March, July and August, flowers are partially open between 0330-0430 h, while during the months of April-June, the flowers remain more or less closed. On the other hand, according to Newstrom *et al.* (1994), a typical flower of *Hemalia patens* opens between midnight at 0200 h and abscisces between 1600 and 1700 h of the same day. The anthers dehisce by longitudinal slits prior to anthesis and at 0200 h, 98% of the anthers dehisce inside the buds which open later (Figs. 2e, f). The number of pollen/anther and per flower is 3510±385 and 17550±510 (x ± SE, n= 10 flowers) respectively. The pollen grains are two-celled, spherical, tricolpate and of 23.5±0.5 μm in diameter with reticulate exine (Figs. 3d, 4e). Pollen viability is 98.5±15.4% (x ± SE, n = 25 flowers). Most individuals present differences in pollen viability between flowers (from 75 to 98%).

In the morning at the time of anthesis, the stigma is still closed and non-receptive. The stigmatic lobes start opening around 1800 h and receptivity is observed until 1630 h of the second day. The flowers are functionally female during second night. The inner surface is copiously papillose, the papillae extending over the margins of two lobes.

During summers (April-June), the anthers in c floral buds and flowers were non-dehiscent exhibited 100% pollen sterility. It was interesti note that stigma from young buds protrude r 3.8±0.1 mm out of the corolla lobes (Fig. 2g, 3e protruding stigmas are bifid and dry, but occasi they became tetrafid (Fig. 3f). The papillae c surface of such stigma were elongated, com arranged (Fig. 4b) and covered with thick undis cuticle pellicle layer (Fig. 4g, h). The protopla the papillae were in highly degenerated form (Fi These failed to support pollen germination (Fi On the other hand, the papillae on the surface of in open flowers were medium sized and loosely ar with thin disrupted cuticle pellicle layer on thei surface (Fig. 4a, f). Their protoplast consisted organized organelles and large vacuoles (Fig. 4f). germinates normally on such surface (Fig. 4c).

H. paten is a self-compatible, non-apomictic s with low fruit production (7%) under natural con In open pollinated flowers only 25% pollen ger on the stigmatic surface. There is no sign difference between the fruit set of self- and pollinated flowers (÷2=1.21, P=0.28). The nu ovules/flower is 43±3.4 (x ± SE, n = 16 flowe pollen-ovule ratio is 408.14:1 indicating fac xenogamy (Cruden 1977). Family Rubiaceae s great variety of breeding systems (Bawa & Beac The rate of pollen tube growth in hand pollin open pollinated pistils and pollen tube is see base of the style 24 h after pollination (Fig. 1 fruits developed from cross-pollination are lar large and heavy seeds as compared to that self-pollination. The plants after natural pollination produce a mean of 54.2 ± 12.4 f SE, n=21). According to Levey (1987), *H* displays temporally multicoloured fruits, as fru in colour from green to cream to pink to red to to black as they ripen over a 4-6 days period Rica. Zurovchak (1997) has observed that re pre-ripe as well as ripe fruits accelerates fruit m rate in *H. patens.*

C. NECTAR — Nectar secretion begin before anthesis and continues even after ant ca. 2 h. During the months of September- Ma open flowers produce 3±1.2 μl nectar and t secreted thereafter amounts to 8±2.1μl. Thus flower secretes a total of 11±2.5 μl nect (glucose, fructose and sucrose) and amino a present in the nectar, while alkaloids, lipids and reducing acids were absent. Galetto (199 made similar observations in *H. paten* concentration at anthesis was 16% and late between 20-25% probably due to evaporation

: nectar secretion was much influenced by perature and in summers (April-July) with the rise temperature, nectar quantity declined and with perature between 37°C and 47°C; nectar was more ıess absent (0.5±0.15μl). Lasso & Naranjo (2003) ·e studied the effect of pollinators (seven ımingbirds) and nectar robbers (1 hummingbird and ›erching birds) on nectar production and pollen ›osition in *Hamelia patens*. Their results have icated that nectar robbers may not affect the foraging ıaviour of hummingbirds, and therefore pollen ›osition.

D. FLORAL VISTORS — The flowers are ited by honey bees-*Apis dorsata* (Fig. 1b) and *A. ica*, butter flies (*Pieris brassicae*-Fig.1a) *and Danaus xippus*), wasps (*Vespa* sp.), ants (*Camponotus npressus*- Figs. 2a, b), house flies (*Musca domestica*-;. 2d), spider (Fig. 2c) and purplerumped sunbirds, :h male (purplish black- Fig. 1e) and female (greenish low- (*Nectarinia zeylonica*- Fig. 1c). It is interesting observe that the butterflies visit in the morning hours 300-1000 h); while the sunbirds are seen in the ernoon between 1500-1700 h. Thomas *et al.* (1986) ve studied the interactions between hummingbirds and tterflies in *Hamelia patens*. Their interesting study s demonstrated that a semi-territorial hummingbird, *pherusa eximia*, guarded a *Hamelia patens* bush ainst butterflies in poor weather. During good weather, ıen butterflies were abundant and active, *E. eximia* andoned the bush. According to Thomas *et al.* (1986), ? net energy balance of chasing away the relatively w butterflies in poor weather was positive, but the lance would have been negative had *E. eximia* tried exclude all butterflies during good weather. The ıtcome of competition between hummingbirds and ıtterflies may depend on the size of the resource being :fended; the availability of alternative food resources; e size, number and activity of butterflies and the eather. Galetto (1998) has also observed hummingbirds siting the flowers.

Interestingly, several squirrels also visit the plants ıring at the time when flowers produce higher quantity nectar (Fig. 1d). However, they eat away the swollen ıse of the flower full of nectar secreted by the nectary :esent at the base of style. The corolla lobes remain pen during the day hours and flowers produce nectar ıroughout the day.

Based on the observations of the present authors on *lamelia patens* plants grown in the gardens of North ıdia and those are found in their natural habitat in ırgentina it is concluded that the reproductive biology f this species exhibit some distinct differences. In ıdia, the distinct male and female phases seen in its atural habitat are lacking. With the increase in temperature in India, pollen fertility, stigma receptivity and their protruding nature and nectar production is reduced. Some floral visitors e.g. hummingbirds are absent from India and those found exhibit different visitation behaviour. Due to these basic differences, this ornamental species fail to show reproductive success comparable to that observed in its natural habitat. It can be further concluded that *Hamelia patens* introduced to India as an ornamental is still in the process of acclimatization.

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