Floral sexuality and breeding system in gum karaya tree, *Sterculia urens*

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Abstract. Comprehensive studies were carried out on phenology, floral sexuality, pollination biology, pollen-pistil interaction, breeding system and fruit and seed set on three populations of gum karaya tree (*Sterculia urens*). The species is andromonoecious and produces a large number of male and a limited number of "bisexual" (functionally female) flowers. The numbers of male and "bisexual" flowers varies not only between trees but also during the flowering period within a tree. Each male flower produces about 5000 fertile pollen grains. Neither in morphology nor in number, is there any difference between pollen grains in the "bisexual" and male flowers. However, pollen grains of "bisexual" flowers are completely sterile and incapable of siring any seeds. Their anthers, however, serve to attract pollinators; the emasculated "bisexual" flowers fail to do so. Thus *S. urens* is apparently andromonoecious but exhibits cryptic monoecy. That the species is self-incompatible was confirmed by controlled pollinations. The self-incompatibility is of the late-acting type and manifests after the entry of the pollen tube into the ovule. *Apis indica* is the only pollinator recorded by us and wind plays no role in pollination. The efficacy of pollination is low as only 56% of flowers were estimated to be pollinated. The pollen load on one-third the number of pollinated stigmas was lower than the number of ovules present. Fruit set under open pollination is poor and is highly variable from tree to tree (0.7–3.2%). Apart from pollination constraint, limited resource availability may also contribute to low fruit set.

Sterculia urens Roxb. (formerly included under the Sterculiaceae is now placed under Malvaceae – Sterculioideae, Alverson et al. 1999, Bayer et al. 1999) is a large to medium-sized deciduous tree up to 20 m in height that yields gum karaya (Indian tragacanth), one of the important non-wood forest products of India. Gum karaya has numerous domestic, food, pharmaceutical, dental, medicinal and industrial uses (Gautami and Bhat 1992, Coppen 1995, Solni 1995) and is a source of income to the tribals and the rural poor. Secretion of the gum requires wounding of the bark (Anonymous 1973, Nair et al. 1995). Owing to steady increase in export demand for gum karaya, *S. urens* is over-exploited. Death of old trees and lack of seedling recruitment in the natural habitats have resulted in large-scale eradication of populations of *S. urens*. In the absence of planned cultivation of karaya trees for gum production, there is a distinct possibility of acute

shrinkage of the once abundant populations of *S. urens*.

Investigations on the reproductive biology including breeding system and seed biology are essential for any rational strategy for its propagation and genetic improvement. To our knowledge there is no published account on any aspects of reproductive biology and seed production in *S. urens*. Even the sexuality of flowers is not clearly documented. Some taxonomic accounts have described *S. urens* as andromonoecious (Cooke 1967, Matthew 1983); others have described the species as polygamodioecious (Ramaswamy and Razi 1973), monoecious (Bhattacharya and Johri 1998) and unisexual flowers (Verma et al. 1993). We have carried out detailed studies on the reproductive biology including breeding system, seed biology and seedling establishment on three populations of *S. urens* growing in the forests of Madhya Pradesh, India. This paper presents the results of our investigations on the phenology, floral biology, pollination biology and breeding system, and fruit and seed set.

Material and methods

S. urens (Malvaceae–Sterculiodeae) is widely distributed in India and occurs in dry, rocky forests in the sub-Himalayan tracts, Gujarat, Rajasthan, Madhya Pradesh, Uttar Pradesh, Maharashtra, Andhra Pradesh and along the West coast in Konkan and North Kanara, (Karnataka State) stretching up to Kerala. The tree is frequently associated with *Boswellia serrata* (salai guggul) another gum-resin yielding tree.

Study sites. Work was carried out for over four years (1994/95–1997/98) on a natural population of 50 trees growing in the forest near Ghatti (referred to as GG site), about 40 km from Gwalior, Madhya Pradesh close to the National Highway Number 3. Some studies were also carried out on populations growing on two other natural stands in the forests of Madhya Pradesh – Shivpuri (28 trees, referred to as SV site) and Sheopur (175 trees, indicated as SH site). For detailed studies 20 trees in GG population and 10 each in SV and SH populations were selected at random and marked.

The marked trees ranged from 8.0 to 17.0 m in height and 0.6–2.4 m in circumference at breast height. The ground cover in the study sites remained dry except during the monsoon season when a large number of annuals and grasses appeared. Other co-existing species with *S. urens* in the study sites are *Acacia catechu*, *A. leucophloea*, *A. nilotica*, *Anogeissus pendula*, *Diospyros indica*, *Phyllanthus emblica*, *Prosopis cineraria*, *Maytenus emarginata*, *Nyctanthes arbor-tristis* and *Zizyphus nummularia*.

Periodical field visits were made to record various phenoevents associated with foliage, flowers and fruits. Mature fruits were harvested before dehiscence and allowed to open in the laboratory at Delhi. Seeds were collected and stored under laboratory conditions.

Floral biology. The number of flower buds/ flowers per inflorescence and the sex of the flowers in an inflorescence (N = 15 per tree) were recorded for each selected tree from randomly chosen branches. The floral parts were studied by using a hand lens.

Pollen production was calculated following the method described by Cruden (1977). Mature undehisced anthers (N = 30) were cleared in 1N NaOH for 1 h at 60 °C, washed in tap water, mounted in 1:1 solution of 1% acetocarmine and 50% glycerine. Each anther was gently squashed to release the pollen. The pollen grains were observed under a microscope and the total number was counted. This number was multiplied with the total number of anthers to calculate pollen production per flower.

Pollen fertility was assessed by staining them in 1% acetocarmine. Pollen viability was estimated by fluorescein diacetate (FDA) test introduced by Heslop-Harrison and Heslop-Harrison (1970). Pollen grains were tested for the presence of starch and lipids using I_2KI and Sudan III, respectively. Pollen nuclei were stained using DNA flurochrome, bis-Benzimide (Hoechst No.33258) (Hough et al. 1985). Pollen grains were mounted in a drop of the flurochrome (20 µg/ml in distilled water) and observed under the fluorescence microscope using UV filter combination (excitation filter 330–380 nm, dichromatic mirror 400 nm and barrier filter 420 nm).

The pistil. Stigma surface proteins were visualized using Coomassie brilliant blue R as described by Heslop-Harrison et al. (1974).

Esterases on the stigma surface were localized by employing the method described by Mattsson et al. (1974), using alpha-naphthylacetate as a substrate and fast blue B as a coupling reagent. Receptivity of the stigma was investigated by studying pollen germination and pollen tube entry into the stigma in manually pollinated pistils using aniline blue fluorescence technique (Linskens and Esser 1957, Shivanna and Rangaswamy 1992).

For cytochemical studies, stigmas and styles of different developmental stages were fixed in 2.5% glutaraldehyde-paraformaldehyde fixative (Karnovsky 1965) prepared in 0.1M cacodylate buffer pH 7.2 for 4 h at 4 °C and dehydrated through a graded series of ethanol (Feder and O'Brien 1968). The dehydrated materials were infiltrated with and embedded in glycol methacrylate monomer mixture. Semi-thin sections (1 or 2 μm) were cut using glass knives and used to localize the various components. The cuticle was localized using 0.02% auramine O (Heslop-Harrison 1977), proteins with Coomassie brilliant blue R (Fischer 1968), insoluble polysaccharides with PAS reagent (McGukin and McKenzie 1958), pectins with alcian blue (Heslop-Harrison 1979), and lipids with auramine O (Heslop-Harrison 1977).

For Scanning Electron microscopy, stigmas from freshly opened flowers and pollen grains from freshly dehisced anthers were fixed in Karnovsky/FAA fixative. They were dehydrated by passing through an ascending series of cold acetone (30–100% for 15 min each), critical-point-dried, mounted on aluminium stubs using double-sided adhesive tape and coated with gold using a sputter coating unit. The stubs were observed using a Leo 435VP scanning electron microscope.

Breeding system. Controlled pollinations were carried out on selected trees at the time of maximum stigma receptivity (3–12 h after anthesis) using pollen grains from freshly dehisced anthers. Inflorescences were selected randomly and tagged. All open flowers and flower buds were removed from the inflorescences; only the "bisexual" flowers one day before anthesis were retained and the inflorescences were bagged. As our repeated studies established that pollen grains from "bisexual" flowers are sterile, emasculation was not required. On the next day (i.e. on the day of anthesis) the bags were removed from the inflorescences and the flowers were pollinated using pollen from freshly dehisced anthers from male flowers (of the same

tree for geitonogamous pollinations and from another tree for xenogamous pollinations). The inflorescences bearing manually pollinated flowers were bagged again. As fruit set resulting from open pollination was very low, larger numbers of manual pollinations were needed for each treatment to obtain dependable results.

Pollinated flowers were observed periodically for fruit set. Some of the pollinated flowers (10–15 for each type) were fixed 48 h after pollination and used for studying pollen germination and pollen tube growth applying the aniline blue fluorescence technique (Shivanna and Rangaswamy 1992).

Apomixis: Eight trees of Ghatti population were tested for the occurrence of apomixis. "bisexual" flowers (N = 150 from each tree) were selected following the procedure described above and bagged without emasculation. At Shivpuri and Sheopur, 175 flowers from 5 trees each were used for this treatment.

Pollinators. Initial studies on floral visitors were made from dawn to dusk for 30 min every hour, on the basis of which subsequent observations were confined to the time frame of 08:30 h to 16:30 h. The number of floral visits made by an insect and the time spent on each flower were recorded using a stopwatch. Insects were trapped while they were foraging the flowers and were killed using ethyl acetate vapours and observed under a stereomicroscope for the presence of pollen grains on their bodies. To determine pollen load on insects (Dafni 1992), they were immersed individually in small screwcap vials (10 ml) containing 3 ml ethanol. The vials were vigorously shaken for 2 min to remove pollen grains from the body of the insects. Insects were taken out from the vial and the suspension was poured on two glass slides. After evaporation of ethanol, pollen grains were mounted in a few drops of glycerine, scanned under a microscope and the number of pollen grains was counted (Dafni 1992).

Role of wind in pollination. A set of 5 isolated trees was used to assess the possible role of wind in pollination during 1994/95 and 1996/97. Glass slides (75 × 25 mm) smeared with glycerine jelly (N = 150) were hung at various heights in and around the canopy. After 48 h of exposure, the slides were collected, stained with auramine O and observed under the fluorescence microscope using UV filter combination for the presence of pollen grains.

Fruit set. The number of fruits formed were studied on plants from the day of pollination until maturation and dehiscence. To estimate flower:-fruit ratio the total number of "bisexual" flowers borne on an inflorescence and the number of mature fruits that developed on it were calculated.

Results

Phenology. Figure 1 presents the details of phenological events. Trees bear leaves only for about three months. Trees are free from leaves during the entire period of flowering and fruiting.
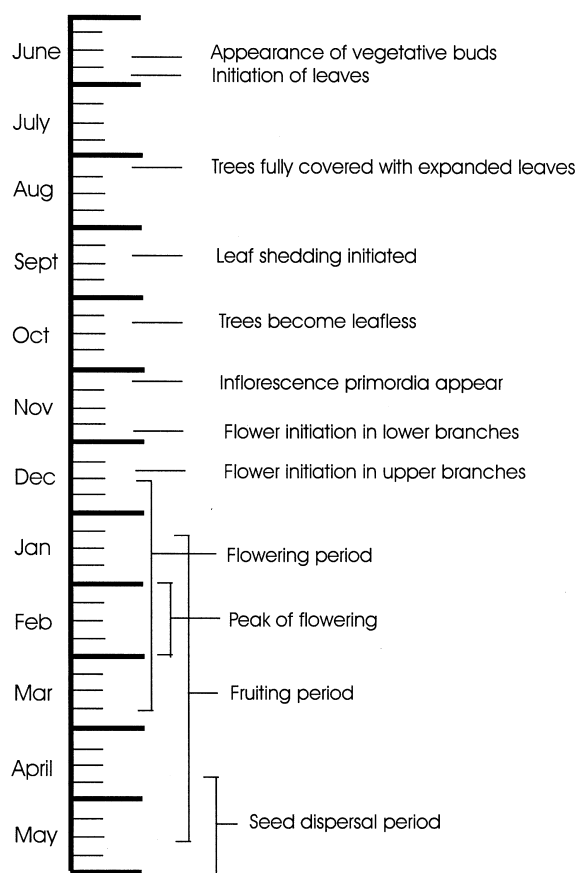

Fig. 1. Various phenoevents in *Sterculia urens*. Note that the trees are clothed with the foliage for about three months commencing with monsoon and become leafless for over eight months in the year. Flowering lasts three months and fruiting period extends over four months

Floral sexuality. The inflorescence is a complex branched panicle, borne at the ends of vegetative branches (Fig. 2A–C). Flowers are arranged in an acropetal succession on each branch of the inflorescence. Both male and "bisexual" flowers are campanulate, greenish-brown in bud condition and turn greenish-yellow at the time of opening and emit an unpleasant odour. The trees are andromonoecious and produce male and "bisexual" flowers. Tree numbers GG 1, GG 5, GG 6 and GG 10 did not bear "bisexual" flowers in any of the four years of study. The number of flowers on each inflorescence ranges from 579 to 3689. At the peak of the flowering season 99 ± 14 flowers open each day in a panicle. The number of "bisexual" flowers when compared to that of male flowers is very low (Table 1). Production of male and "bisexual" flowers during the entire flowering period in 1996/97 (Table 1) and at the peak of flowering (Table 2) over three years of study (1995, 96, 97) was estimated for 20 trees of Ghatti population (GG 1- 20). The number of male and "bisexual" flowers varied not only from tree to tree but also during the flowering period in the same tree. The proportion of "bisexual" flowers ranged from as low as 0.3% (GG 17) to 7.8 (GG 2). The male flowers as well as the "bisexual" flowers that fail to set fruits abscise 4–6 days after anthesis.

Male flower. Male flowers are pedicellate and are 6.5 ± 0.29 mm long (N = 30) at anthesis. The perianth is greenish-yellow, and is made up of five united tepals that are densely covered with glandular trichomes on both the surfaces. There are 10 stamens (Fig. 2D) and the filaments are fused at the base to form an androphore. Vestigial carpels are not visible in male flowers. Anthers are dithecous and are bent outwards at anthesis. The male flowers open between 09:00 and 11:30 h on sunny days and between 12:00 and 02:00 h on cloudy days. The anthers dehisce 1–2 h after anthesis (flower opening) and shed their pollen soon after (Fig. 2E).

Pollen grains in male flowers are ellipsoidal, trizonocolporate with a reticulate

surface ornamentation (Fig. 2F). They are 26.67 ± 0.59 μm in diameter. On an average each male flower produces 5160 ± 1141 pollen grains. They show abundant lipids (in response to Sudan III) only in the pollenkitt material present on the surface. The pollen grains are 2-celled at the time of shedding. Pollen grains are rich in starch as revealed by their staining response with I_2KI solution (Fig. 2G).

A high proportion of pollen grains ($86.75 \pm 5.89\%$) was fertile as tested through acetocarmine staining (Fig. 2I). Pollen from freshly dehisced anthers showed 83.85% viability as determined by the FDA test (Fig. 2H). There was no significant difference ($p = 0.05$) in the viability of pollen in the three populations studied. The viability steadily decreased after 6 h under laboratory conditions. In all three populations pollen viability was completely lost 9 days after shedding.

"Bisexual" flower. "Bisexual" flowers are pedicellate and 6.8 ± 0.11 mm long ($N = 30$) at anthesis. The perianth is greenish-yellow and is made up of five tepals united at the base (Fig. 3A). They are covered with numerous glandular trichomes. There are 10 stamens (Fig. 3B) present in a ring around the carpels. The lower parts of the filaments are fused at the base to form a staminal sheath, which surrounds the lower one third of the ovary. A distinct gynandrophore (fused parts of gynoecium and androecium) is clearly visible. Anthers do not dehisce unless there is some mechanical disturbance. The "bisexual" flowers are generally located at the base of the inflorescence branches and are the first to open.
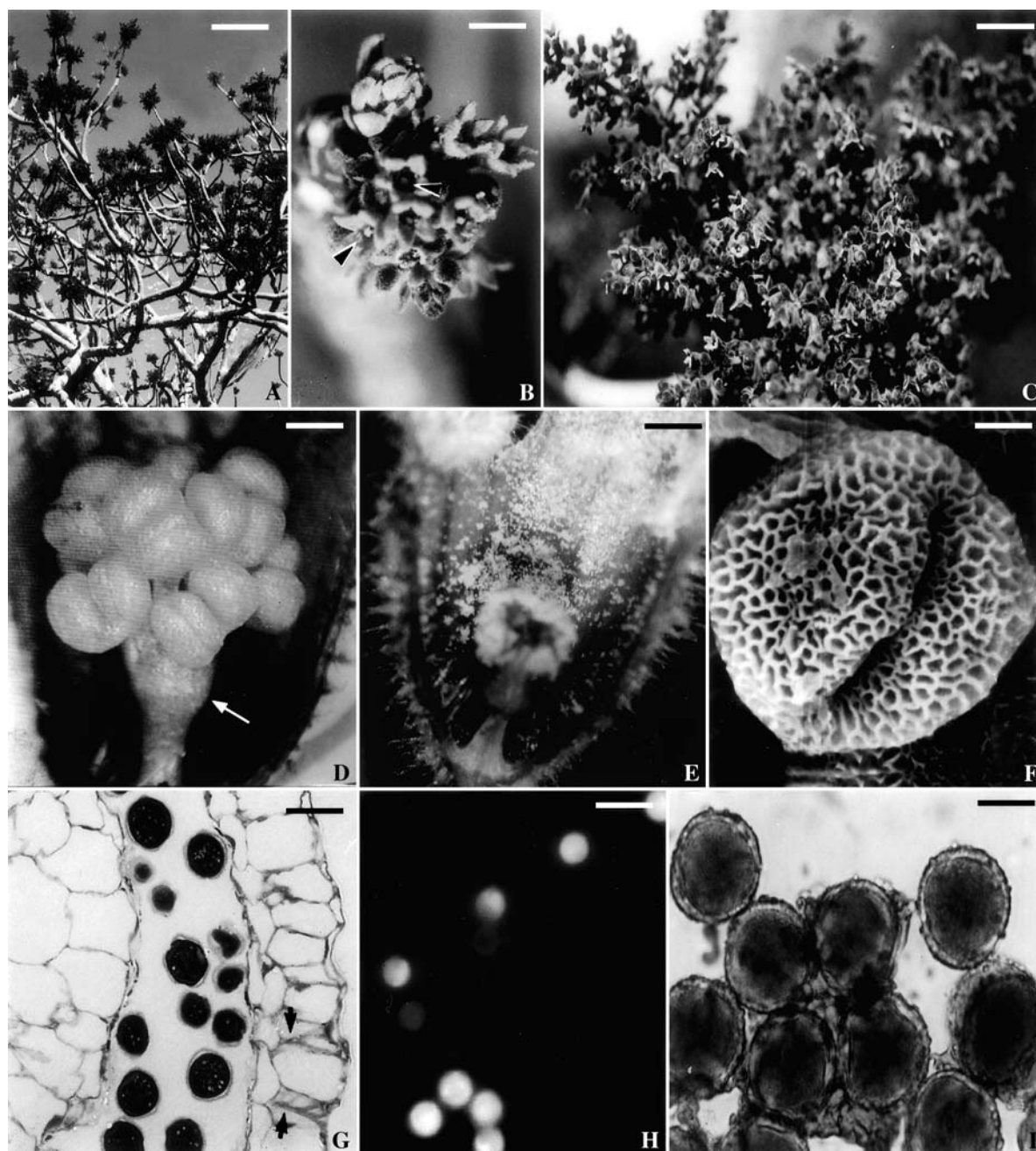
Morphologically, there is hardly any difference between the pollen grains borne in "bisexual" flowers from those of male flowers. Pollen grains from "bisexual" flowers are 24 ± 1.32 μm in diameter. In wholemount preparations and sections they appear almost empty (Fig. 3C, D). There is no difference in the number of pollen grains produced in anthers of the two whorls. The number of pollen grains in "bisexual" flowers ($4987 \pm$ 1079 per flower) is not markedly different from that of male flowers.

Although pollen grains from "bisexual" flowers appear morphologically normal, they do not take up acetocarmine stain (Fig. 3C). FDA test also showed that the pollen grains are non-viable. Further, pollen grains from "bisexual" flowers failed to germinate on the receptive stigma following controlled pollinations.

To identify the stage of pollen sterility, details of pollen development were studied in male and "bisexual" flowers. Developmental details of pollen in male and "bisexual" flowers were identical until the formation of microspore tetrads. In male flowers, the secretory tapetum shows initiation of degeneration during the tetrad stage. The microspores are released from the tetrads by dissolution of the callose wall. Following the division of the microspore the tapetum degenerates completely and the hypodermal layer develops into the endothecium, in which the fibrous thickenings appear by the time pollen grains reach maturity. In "bisexual" flowers, the tapetal cells, instead of showing signs of degeneration during the tetrad and early microspore stages, enlarge and accumulate insoluble polysaccharides. The tapetal cells persist for a longer time in "bisexual" flowers than those in the male flowers and degenerate only after the microspore mitosis. The mature non-fertile pollen grains are highly vacuolated. A majority of pollen grains do not contain any reserve materials (Fig. 3D); only about 30% show the presence of a few starch grains.

The pistil is 2.4 ± 0.2 mm long at anthesis, and consists of well-demarcated stigma, style and ovary. The stigma is five-lobed and occupies the mid level of the perianth tube at the time of anthesis (Fig. 3A, B). The stigma lobes show a slight downward curvature one day after anthesis. Although the stigma appears dry under the light microscope, a thin and irregular secretion is seen under the scanning electron microscope (Fig. 3E). Proteins and non-specific esterases were localized on the surface of the stigma at the time of anthesis. The esterase activity was very low in

stigmas 2–3 days before anthesis; the activity was maximum at the time of anthesis.

Longitudinal sections of the stigmatic lobes at anthesis showed two distinct zones – the upper secretory zone and the lower cortical zone. The cells of the secretory zone were densely cytoplasmic and the interstices of the cells were filled with proteins, insoluble poly-saccharides and lipids. The cortical cells were compactly arranged and vacuolated. The thin exudate on the stigmatic region could be seen under high magnification and the exudate also stained for proteins (Fig. 3F), insoluble poly-saccharides and lipids.

The style is short (1.0 ± 0.06 mm), clearly defined and consists of five ridges, each asso-

Fig. 2. Floral biology of *Sterculia urens*. **A** A portion of the canopy showing dense clusters of inflorescences at the ends of branches. Bar 2ft. **B** A young inflorescence branch with a cluster of "bisexual" flowers. The stigma is clearly visible in many of the flowers (arrowhead). Bar 8 mm. **C** A part of the fully developed inflorescence branch. Most of the open flowers are male. Bar 12 mm. **D** A male flower just before anther dehiscence. Two tepals have been removed to show details of stamens. Arrow indicates androphore. Bar 0.7 mm. **E** As in C but after anther dehiscence. A large amount of pollen has been deposited on the inner surface of the tepals. Bar 1 mm F–H. Pollen grains from male flower. **F** Scanning electron micrograph of a pollen grain. Bar 4.2 μm. **G** A part of a transection of a mature anther lobe stained with I_2KI before dehiscence to show turgid pollen grains filled with reserve materials. Bar 12 μm. **H** Fluorescence micrograph of pollen grains stained with FDA. Pollen grains are viable as revealed through bright fluorescence. Bar 42 μm. **I** Pollen grains stained with acetocarmine, all pollen grains have taken up the stain. Bar 30 μm

ciated with a stigmatic lobe (Fig. 3G). The style is solid with a central core of transmitting tissue surrounded by a parenchymatous cortex. The cells of the transmitting tissue are compactly arranged and do not show any intercellular spaces (Fig. 3H). There is a progressive increase in the amount of starch grains present in the cells of the transmitting tissue which reaches its maximum in the mature pistil.

The gynoecium is pentacarpellary and apocarpous. Each carpel bears 6.03 ± 1.02 ovules

Table 1. Number of male and "bisexual" flowers per inflorescence in different trees of the Ghatti population in the flowering period during 1996–97 (N = 15 inflorescences per tree)

Tree No.	Number of flowers*					
	December		January		March	
	male	"bisexual"[†]	male	"bisexual"[†]	Male	"bisexual"[†]
GG 1**	1599	0	3268	0	1354	0
GG 2	2345	78 (3.2)	3209	102 (3.0)	1769	78 (4.2)
GG 3	1534	12 (0.7)	1765	23 (1.2)	958	21 (2.1)
GG 4	1946	0	2837	12 (0.4)	1893	0
GG 5**	1875	0	2830	0	1875	0
GG 6**	2678	0	2654	0	897	0
GG 7	1589	59 (3.5)	2586	34 (1.2)	2659	12 (0.4)
GG 8	1569	0	1985	4 (0.2)	1764	0
GG 9	1789	23 (1.2)	2376	32 (1.3)	1005	0
GG 10**	1984	0	2352	0	579	0
GG 11	678	0	1216	32 (2.5)	1053	2 (0.1)
GG 12	1278	0	945	56 (5.5)	820	0
GG 13	980	0	1342	83 (5.8)	956	3 (0.3)
GG 14	1752	21 (1.1)	1278	72 (5.3)	1009	0
GG 15	1784	41 (2.2)	1254	25 (1.9)	890	0
GG 16	990	0	1365	33 (2.3)	768	9 (1.0)
GG 17	2820	11 (0.3)	3005	9 (0.2)	879	0
GG 18	2893	30 (1.0)	2643	6 (0.2)	853	0
GG 19	678	4 (0.5)	1965	45 (4.4)	1104	0
GG 20	867	7 (0.8)	1859	43 (4.7)	990	0

* Includes flower buds, open flowers, and developing fruits.
** Did not produce "bisexual" flowers in any of the three years of study.
[†] Figures in parenthesis show the percentage of "bisexual" flowers.

Table 2. Number of male and "bisexual" flowers per inflorescence in different trees of the Ghatti population during the peak of flowering period in February (N = 15 inflorescences per tree)

Tree No.	Total number of flowers*					
	1995		1996		1997	
	male	"bisexual"[†]	male	"bisexual"[†]	Male	"bisexual"[†]
GG 1**	2098	0	1991	0	2419	0
GG 2	3012	198 (6.1)	2059	175 (7.8)	2850	167 (5.5)
GG 3	3456	58 (1.6)	3045	35 (1.1)	2010	27 (1.3)
GG 4	1987	75 (3.6)	1850	0	2871	89 (3.0)
GG 5**	3110	0	2569	0	2691	0
GG 6**	2099	0	2750	0	2953	0
GG 7	3211	175 (5.1)	3091	179 (5.4)	3015	150 (4.7)
GG 8	2567	100 (3.7)	2873	48 (1.6)	2655	29 (1.0)
GG 9	3129	109 (3.3)	3689	131 (3.4)	2950	76 (2.4)
GG 10**	2547	0	2757	0	3011	0
GG 11	3127	58 (1.8)	3015	0	1198	14 (1.1)
GG 12	4003	112 (2.7)	2991	159 (5.0)	3019	101 (3.2)
GG 13	1987	107 (5.1)	1589	129 (7.5)	3151	124 (3.7)
GG 14	3245	48 (1.4)	2719	48 (0.1)	2461	36 (1.4)
GG 15	2345	59 (2.4)	3451	51 (1.4)	2815	61 (2.1)
GG 16	2348	45 (1.8)	2812	0	1855	13 (0.6)
GG 17	2783	34 (1.2)	3031	12 (0.3)	1957	19 (0.9)
GG 18	2890	13 (0.4)	2190	0	1585	14 (0.8)
GG 19	2671	128 (4.5)	2871	119 (3.9)	2819	84 (2.8)
GG 20	3245	95 (2.8)	2991	95 (3.0)	3501	110 (3.0)

* Includes flower buds, open flowers, and developing fruits.
** Did not produce "bisexual" flowers in any of the three years of study.
† Figures in parenthesis refer to the percentage of "bisexual" flowers.

in each locule (N = 30). The entire surface of the ovary is covered with glandular trichomes.

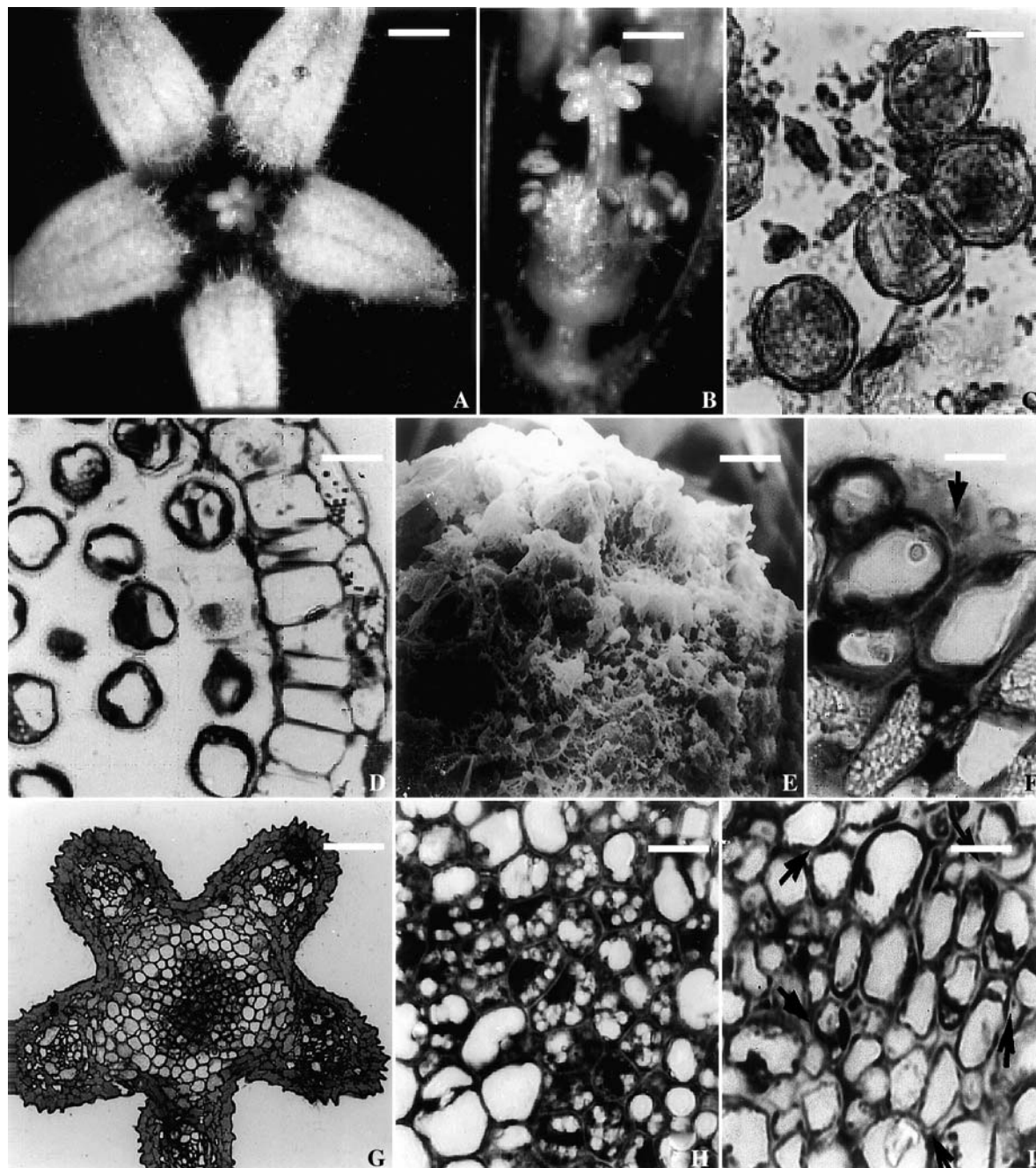**Stigma receptivity.** The receptivity of the stigma was assessed by carrying out manual xenogamous pollinations on stigmas of flowers (implanted in Petri plates containing 1% agar) of different ages (48 h before anthesis to 48 h after anthesis). The pistils were processed 36 h after pollination for studying pollen germination through aniline blue fluorescence. There

Fig. 3. Floral biology of "bisexual" flowers of *Sterculia urens*. **A** Top view of the flower to show five tepals and the five-lobed stigma. Bar 1 mm. **B** Side view of the same stage as in A. Two tepals have been removed to show gynandrophore, details of the stamens and the pistil. Stamens have not dehisced. Bar 0.5 mm. **C** Pollen grains stained with acetocarmine; they have stained poorly indicating that they are sterile. Bar 8.5 µm. **D** Transection of anther lobe. Pollen grains are highly vacuolated. Bar 30.6 µm. **E** A portion of the scanning electron micrograph of stigma surface covered with a lining of dry exudate. Bar 20 µm. **F** A part of the longitudinal section of a stigmatic lobe stained with Coomassie brilliant blue R to show proteinaceous nature (arrowhead) of the exudate. Bar 7 µm. **G** Transection of a mature style stained with toluidine blue O. A central core of transmitting tissue and five radiating arms each with a vascular bundle are clearly seen. Bar 29 µm. **H** A portion of the transmitting tissue of mature pistil stained with Coomassie brilliant blue R to show absence of intercellular spaces. Bar 8 µm. **I** Transection of pistil 3 h after pollination stained with toluidine blue O. Note development of intercellular spaces filled with extra-cellular matrix. Bar 1.6 µm

was no pollen germination on the stigmas in flower buds 48 h and 24 h before anthesis. Stigmas at anthesis and later stages supported pollen germination. Maximum pollen germination (78.5%) was observed in stigmas 3 h after anthesis; this stage also showed maximum number of pollen tubes entering the stigma. The stigma continued to remain receptive for 12 h beyond which there was a gradual decline. On the basis of these studies, flowers that showed good stigma receptivity 3–12 h after anthesis were used for all manual pollinations.

Pollen germination and pollen tube growth in vivo. Manual in vivo xenogamous pollinations on receptive stigmas were carried out and the pistils were processed for studying tempo-

ral details of pollen germination and pollen tube growth through aniline blue fluorescence. Pistils processed 3 h after pollination showed germination of about 20% pollen. Pollen germination increased to 62% after 5 h of pollination and to about 75% seven hours later. There was no further increase beyond 7 h. The pollen tubes were confined to the stigma 3–5 h after pollination and the pollen tubes had just entered the upper portion of the style by 8 h after pollination. Pollen tubes reached the base of the style by 14–18 h after pollination and entered the ovules by 25 h following pollination.

As pointed out earlier, the transmitting tissue of the mature pistil lacks intercellular spaces. The path of pollen tube growth in the transmitting tissue was studied through transections of the style of pollinated pistils. Interestingly, the transmitting tissue developed intercellular spaces after pollination but before the pollen tubes reached the style. Intercellular spaces could be observed as early as 3 h after pollination (Fig. 3I) when the pollen tubes were still confined to the stigma. Thus formation of intercellular spaces was dependent on pollen germination as no intercellular spaces could be detected in the transmitting tissue of the style 1 h after pollination by which time pollen grains had not germinated. The intercellular spaces were filled with proteins, insoluble polysaccharides and lipids. Pollen tubes grew through this extra-cellular matrix (ECM). The amount of starch grains in the transmitting tissue decreased with the passage of pollen tubes as compared to the condition in unpollinated pistils indicating that carbohydrates have been utilized by the growing pollen tubes.

Breeding system. *Apomixis:* None of the bagged "bisexual" flowers produced fruits; all the flowers abscised in 2–3 days. The results were similar in all three populations confirming absence of apomixis in *S. urens*.

Geitonogamous and xenogamous pollinations were carried out and pollen germination and pollen tube growth as well as fruit set were analyzed to establish the breeding system.

Self-incompatibility: Manual geitonogamous self-pollinations and xenogamous cross-pollinations were carried out to ascertain whether or not the species is self-incompatible. Field pollinations were carried out on trees of the GG population during the two flowering seasons (1995/96 and 1996/97) whereas the studies were confined to one year on SV (1995/96) and SH (1996/97) populations.

Pollen germination occurred in both geitonogamous and xenogamous pollinations. Pollen tubes grew through the style (Fig. 4A,B) and entered the ovules (Fig. 4C) in both types of pollinations. However, none of the geitonogamously pollinated flowers set fruits. Flowers on which xenogamous pollinations were carried out set fruits. These studies clearly showed that *S. urens* is self-incompatible, and therefore, an obligate outbreeder.

Fruit set. The fruit set under open pollination was very low and ranged between 0.7 to 3.2% in the Ghatti population (Table 3). Table 3 also gives the data on percent fruit set in manually pollinated flowers on some of these trees for comparison. Fruit set through open pollination was significantly lower (at $p = 0.05$) when compared to that through manual xenogamous pollinations in all the trees studied. In general, fruit set (Fig. 4D) in manually pollinated flowers varied among individual trees. The highest fruit set of 15.7% was recorded on SV 12 growing at Shivpuri and the lowest fruit set (6%) was recorded in SH 10 at Sheopur. The percentage of fruit set in manual pollinations ranged from 8.2 to 14.0 in the Ghatti populations (Table 3), and from 8 to 15.7 and 6 to 14 in the SH and SV populations, respectively.

The flower:fruit ratio was very high and varied from tree to tree. The number of "bisexual" flowers per inflorescence ranged from 33 to 425. The average flower:fruit ratio during 1997 in different trees varied from 11:1 to 472:1.

The length of the ovary at the time of pollination was 1.5 mm. It showed a slight enlargement in three days after pollination. The fruit reached a length of 2.0 cm in 8 days.
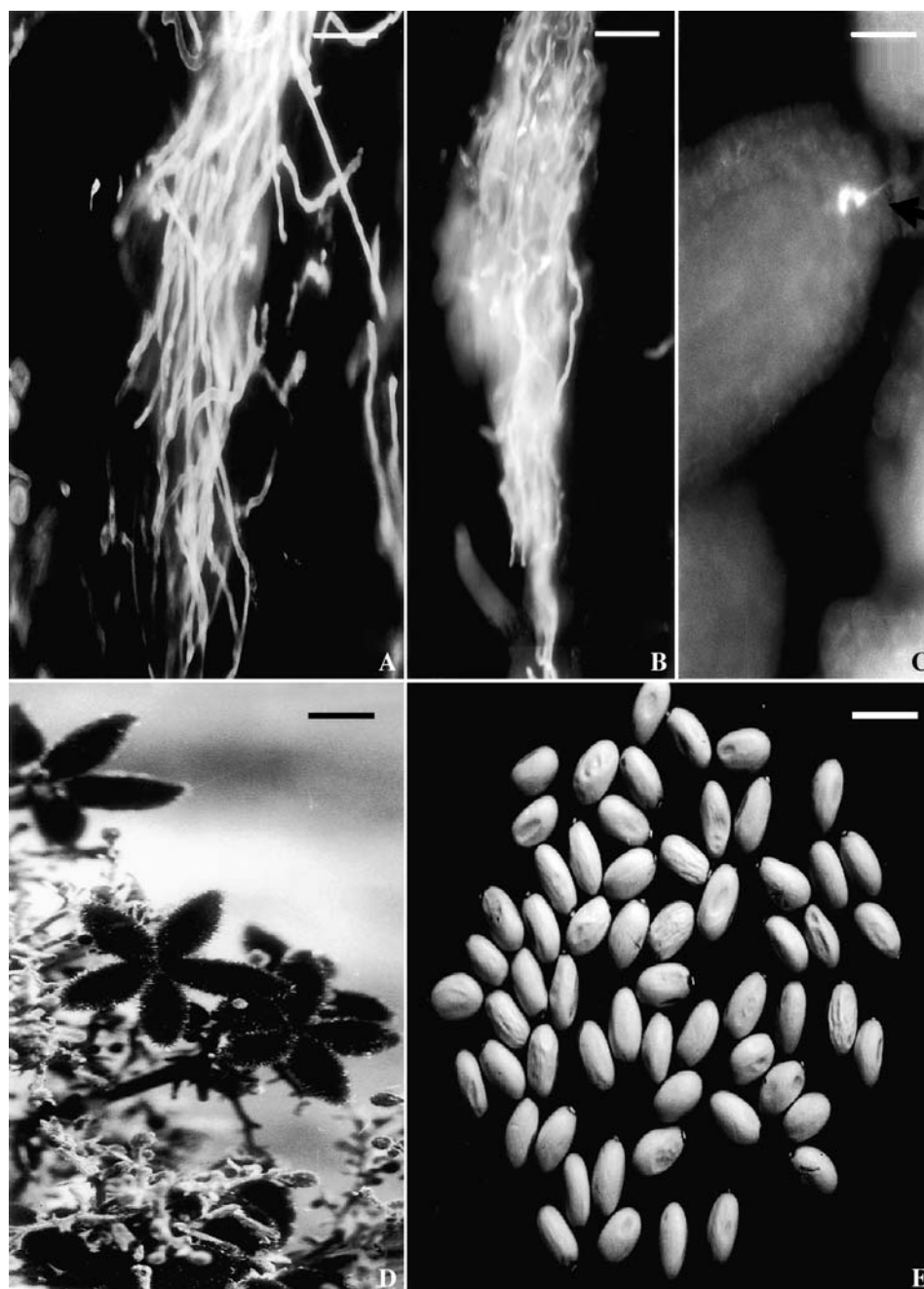
Fig. 4. Pollen tube growth, and fruit and seed set of *Sterculia urens*. **A, B** Aniline blue fluorescence micrographs of xenogamous cross-pollinated (A) and geitonogamous self-pollinated (B) styles. Good pollen tube growth in both styles is obvious. Bars 49 μm (A), 77 μm (B). **C** Entry of pollen tube into the ovule (arrowhead) in self-pollinated pistil. Bar 44 μm. **D** A portion of the inflorescence to show developing fruits on a branch. **E** A sample of mature seeds removed from one of the trees. Bar 8.5 mm

Further growth of the fruit was rapid; it gained another 2 cm in the next 10 days. During the initial stages of development, the fruit wall is velvety and densely covered with a copious amount of soft, reddish-magenta trichomes. As the fruit reaches maturity, it appears star-

Table 3. Fruit set under open pollination during 1996–97 in the Ghatti population (N = 10 inflorescences per tree)

Tree No.	Total No. of "bisexual" flowers per inflorescence	Number of fruits developed	Percentage of fruit set*
GG 1	0	0	0
GG 2	425	4	0.9 (10.0)
GG 3	83	0	0 (11.7)
GG 4	89	2	2.2 (11.5)
GG 5	0	0	0
GG 6	0	0	0
GG 7	255	3	1.2 (8.2)
GG 8	33	1	3.0
GG 9	131	1	0.7
GG 10	0	0	0
GG 11	48	0	0
GG 12	157	5	3.2 (13.8)
GG 13	210	4	1.9 (9.6)
GG 14	129	1	0.7 (14.0)
GG 15	127	1	0.7 (14.0)
GG 16	55	0	0
GG 17	39	0	0
GG 18	50	0	0
GG 19	152	2	1.3 (14.0)
GG 20	160	3	1.8 (10.6)

* Figures in parenthesis represent fruit set in manually pollinated flowers (N = 130–200 per tree) on the same tree.

shaped bearing the spread out follicles, each covered with hard and stiff trichomes. Each follicle attained its maximum length (6.8 ± 0.8 cm) in 40 days from the day of pollination. The mature fruit is a dry dehiscent follicle covered with dense, rough and pointed trichomes.

The majority of trees bore red fruits. However, five out of 50 at GG population bore green fruits. The colour of the fruit was constant for each tree over the four years of observation, indicating that fruit colour is genetically controlled. In the other two populations (SH and SV) all trees bore red fruits.

Out of total of 30 ovules borne in each ovary, 8–26 ovules developed into seeds (Fig. 4E) each with a solitary embryo. There was considerable variability between trees regarding seed size (9 × 5 mm to 11 × 6 mm). The 100 seed weight ranged from 15.8 to 21.7 g.

Pollination efficiency in natural habitat. To understand the possible reasons for poor fruit set under open pollinations, pollination efficiency was investigated by screening a large number of stigmas two days after anthesis under the microscope for the presence of pollen. Of the 1048 flowers examined from the Ghatti population, pollen grains were present only on stigmas of 56% of the flowers; the remaining 44% of the stigmas did not have pollen grains. Amongst the stigmas that contained pollen grains, 36% showed more than 30 pollen grains each (equal to the number of ovules) and the remaining 20% indicated less than 30 pollen grains. The pollination efficiency in the SV and SH populations was also similar to that in the GG population.

Pollination agents. The flowers of *Sterculia urens* do not produce nectar (Vogel 2000). Anthers of both the flower types are bright

yellow, produce abundant pollen and might serve to attract insects. Thus pollen grains appear to be the only floral reward for the visitor. Since pollen grains produced by the male flowers are rich in starch and lipids, their food value to the visitor is high. Careful and prolonged observations showed limited insect activity even during the peak season of flowering.

Apis indica was the only floral visitor observed. It visited both male and "bisexual" flowers on the day of anthesis. The average duration of one visit by the bee to a flower was 4.0 ± 1.7 s (N = 16). Foraging activity starts around 10:00 h in the morning and reaches its maximum between 11:30 and 12:30 h. There was hardly any insect activity in the afternoon. The bees were collected while they were foraging the flowers and were examined under the stereomicroscope for the presence of pollen on their body parts. Pollen load of 412 ± 15 was found distributed on the head, thorax and abdomen of the bees.

To determine whether or not the anthers of "bisexual" flowers are responsible for the attraction of pollinators, 187 "bisexual" flowers were carefully emasculated without disturbing the tepals and observed for the visits by bees. No bee visit was recorded in 184 flowers. Bees called on only three emasculated flowers probably as they happend to be located close to the un-emasculated "bisexual" flowers.

The possibility of wind pollination was studied by hanging slides smeared with glycerine jelly (vertically and horizontally) at various heights by means of threads in and around the canopy of the trees and examining them for the presence of pollen grains after 48 h. No pollen grains were found on any of the slides hung vertically or horizontally.

Discussion

Cryptic monoecy. Our studies have clearly shown that *Sterculia urens* is andromonoecious and produces a large number of male and a small number of "bisexual" flowers.

According to Janzen (1977) production of numerous male flowers in andromonoecious system increases the conspicuousness of the flowers and thus improves the pollination efficiency of the "bisexual" flowers. Another advantage of an andromonoecious system (in which pollen is packed into many small flowers) is that it extends the period of pollen availability (Harder and Thomson 1989). Andromonoecy is widely distributed in both insect- and wind-pollinated species (Lloyd 1979, Primack and Lloyd 1980, Bawa and Beach 1981, Thomson and Barrett 1981). Four main hypotheses have been proposed for the evolution and maintenance of andromonoecy: a) reduction of inbreeding, b) increase of pollen deposition per stigma, c) decreased predation of ovaries and fruits and d) optimization of the allocation of resources for reproduction (Bertin 1982a, Anderson and Symon 1989, O'Brien 1994). Many studies support the hypothesis that andromonoecy optimizes allocation of resources for both male and female function (Bertin 1982b, Soloman 1986, O'Brien 1994).

Except for a slight reduction in size, the anthers and the pollen grains of "bisexual" flowers of *S. urens* appear apparently normal. However, detailed studies carried out through (1) acetocarmine staining, (ii) fluorescein diacetate test and (iii) pollination of stigmas clearly showed that the pollen grains of "bisexual" flowers are sterile. Thus, morphologically "bisexual" flowers are functionally females, and in effect *S. urens* exhibits cryptic monoecy. Anatomical studies of anthers of "bisexual" flowers indicated abnormalities in the tapetal tissue as reported in many species characterized by nuclear or cytoplasmic male sterility (see Shivanna and Sawhney 1997, Shivanna 2003). The tapetal cells in "bisexual" flowers enlarge and accumulate carbohydrates in the form of starch and other insoluble polysaccharides and persist longer than those from male flowers. Pollen grains from "bisexual" flowers accumulate very few starch grains as compared to those in male flowers. These observations suggest that pollen sterility in

"bisexual" flowers is mediated through lack of mobilization of energy sources from the tapetum to the pollen grains as has been reported in some male sterile systems (Shivanna and Johri 1985).

The evolutionary significance of anther and pollen development in "bisexual" flowers of *S. urens* is not clear. Several investigators have suggested that the non-viable pollen in the pistillate flowers may act as rewards for pollinators (Kaplan and Mulcahy 1971, Bawa and Beach 1981, Sullivan 1984). Non-functional pollen grains have been reported in many other androdioecious species particularly in species whose flowers lack nectar and are pollinated exclusively by bees (Cane 1993). However, in the cryptically dioecious *Thalictrum pubescens*, anthers in "bisexual" flowers do not seem to perform the function of vector attraction (Davis 1997). In *S. urens* the anthers of "bisexual" flowers are as colourful as those of male flowers, the flowers are nectarless and bees are the exclusive pollinators. Results of our studies on emasculation of "bisexual" flowers have clearly shown that anthers do function as pollinator attractants. Many studies have highlighted the role of pollen odour in attracting insects (Dobson 1988). In *S. urens* it is not clear whether it is the colour of anthers visible from the top of the open flower or the pollen odour which is involved in insect attraction.

Sex-expression in andromonoecious species is widely variable among individuals within populations (Emms 1993, Diggle 1993, O'Brien 1994). In *Solanum hirtum* sex expression has been shown to be plastic even among individuals of the same genotype; this plasticity is at least partly determined by the resource status of the plant (Diggle 1993). In *S. urens* also, the proportion of "bisexual" flowers between the trees ranged from 0–7.8% (Tables 1 and 2). There was a positive correlation between the number of "bisexual" flowers and the number of fruits produced in a particular tree. Only those trees that produced a larger number of "bisexual" flowers were found to be better fruit setters than the others. Thus, the extent of resource allocation

to fruit set seems to be determined right at the time of flower initiation.

Several trees of the GG population did not produce "bisexual" flowers at all during some years of the study. Four trees of this population did not produce "bisexual" flowers and therefore fruits throughout the four years of study. Thus, these four plants were in essence male. Whether this is a feature of phenotypic plasticity of andromonoecy or a beginning of the establishment of androdioecy is not clear (see also Symon 1979, Ross 1982, Thomson et al. 1989).

Pollen-pistil interaction. The structural features of the stigma and the composition of the stigmatic surface components are similar to those reported for other genera (Heslop-Harrison and Shivanna 1977, Shivanna and Johri 1985, Shivanna 2003). The style of *S. urens* is solid. In all other solid-styled systems reported so far (Raghavan 1999, Shivanna 2003), the transmitting tissue invariably contains intercellular spaces filled with extracellular matrix (ECM). Pollen tubes grow through the ECM and there is evidences that the ECM is actively involved in pollen tube growth (Cheung 1996, Raghavan 1999, Shivanna 2003). A unique feature of the style in *S. urens* is the absence of intercellular spaces in the transmitting tissue in the mature pistil. Pollination is known to induce many changes in the style and the ovary in several plants (Cheung 1996). These include changes in enzyme profiles, transcription and translation activities in the style and ovary, degeneration of one of the synergids, and initiation of ovule development in many orchids (Shivanna 2003). As these changes are initiated before the arrival of the pollen tubes in the style/ovary, it is believed that a pollination stimulus reaches the style and ovary much before the arrival of pollen tubes. The precise nature of the pollination stimulus is not understood. Interestingly, in *S. urens* pollination induces development of intercellular spaces filled with ECM in the transmitting tissue of the style making it conducive for pollen tube growth. As intercellular spaces develop within 3 h after pollination by which time pollen tubes are still confined to the

stigma surface, the development of intercellular spaces appears to be the result of transmission of the pollination stimulus into the style.

Breeding system. Numerous studies have shown that outcrossing is a very significant reproductive strategy in tropical trees (Zapata and Arroyo 1978, Frankie et al. 1983, Baker et al. 1983). A large number of tropical trees bearing "bisexual" flowers are self-incompatible (Bawa 1992). Studies on pollen germination and pollen tube growth in both geitonogamous self- and xenogamous cross-pollinations as well as fruit set in *S. urens* showed that it is self-incompatible. This is an important adaptation to prevent inbreeding since the number of male flowers is disproportionally large and there is ample chance for geitonogamy. However, the inhibition of self-pollen tubes is delayed until their entry into the ovule. Such type of incompatibility has been categorized as late-acting self-incompatibility (LSI) or ovarian SI (see Seavey and Bawa 1986 for a comprehensive discussion). LSI is quite common and has been reported in many tree species (Bawa et al. 1985, Gibbs et al. 1999, Lewis and Gibbs 1999) including *Sterculia chicha* (Taroda and Gibbs 1982).

Pollination efficiency and fruit set. In *Sterculia urens*, fruit set is generally very low, although it varied markedly among individual trees. Many hypotheses have been put forward to explain low fruit set. According to Bawa and Webb (1984), lack of successful pollination, adjustment of maternal investment to match available resources, and inherent conflicts in optimization of male and female reproductive success are some of the reasons for low fruit set.

A careful examination of more than 1000 "bisexual" flowers of *S. urens* collected two days after anthesis showed that 44% of the stigmas were unpollinated and in one third of the pollinated stigmas, the number of pollen grains deposited on the stigma was less than the number of ovules present in the ovary. As the proportion of geitonogamous and xenogamous pollen in open-pollinated pistils could not be determined, it is presumed that the number of flowers that receive sufficient number of xenogamous pollen are likely to be lower than recorded. Thus, pollination is a major constraint for fruit set in *S. urens*.

Field observations showed that anemophily does not occur in *S. urens*. Although only *Apis indica* was identified as the pollinator, the number of visits it made was rather low. Absence of nectar in the flowers could be one possible reason. Another reason could be the competition from *Boswellia serrata*, which co-exists with *S. urens* in all the study sites. *S. urens* and *B. serrata* are the only two species that come to flower simultaneously during December-March in all the study sites. The flowers of *B. serrata* are "bisexual", more conspicuous because of their bright colour, contain a considerable amount of nectar and are also pollinated by *A. indica*. Unlike in *S. urens*, pollination efficiency in *B. serrata* is almost 100% (Sunnichan, unpublished).

Although pollination is a major constraint for low fruit set in *S. urens*, it is not the only limitation; even in manually pollinated flowers the fruit set never exceeded 16%, indicating that there are additional restrictions involved for fruit set. In the absence of leaves during the entire reproductive phase, trees have to depend on the limited reserve materials stored in the stem for fruit set. Thus availability of resources is likely to be a major constraint for fruit set in *S. urens*.

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