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Full Length Research Paper

Floral biology and the effects of plant-pollinator interaction on pollination intensity, fruit and seed set in *Solanum*

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Reproductive biology and patterns of plant-pollinator interaction are fundamental to gene flow, diversity and evolutionary success of plants. Consequently, we examined the magnitude of insect-plant interaction based on the dynamics of breeding systems and floral biology and their effects on pollination intensity, fruit and seed set. Field and laboratory experiments covering stigma receptivity, anthesis, pollen shed, load and viability, pollinator watch vis-à-vis controlled self, cross and pollinator-exclusion experiments were performed on nine taxa of *Solanum*: *Solanum aethiopicum* L., *Solanum anguivi* Lam., *Solanum gilo* Raddi, *Solanum erianthum* Don, *Solanum torvum* SW, *Solanum melongena* L. ('Melongena' and 'Golden') and *Solanum scabrum* Mill. ('Scabrum' and 'Erectum'). Pollen shed commenced 30 min before flower opening attaining peak at 20 to 30 min and continued until closure. Stigma was receptive 15 to 30 min before pollen release, making most species primary inbreeders (100% selfed) but facultatively outbreeding (12.5 to 75%) through insect pollinators such as *Megachile latimanus*, *Diplolepis rosae* and *Bombus pennsylvanicus*. *S. scabrum* 'Scabrum' was an obligate inbreeder, while *S. scabrum* 'Erectum' was facultatively outbreeding (12.5%). *S. melongena* 'Melongena' was strongly outcrossing (75%) than its relative 'Golden' (25%). Small pollen and anther assured high pollen load and pollination efficiency and vice versa, except *S. torvum*. Diploid species (2n = 24) received crossed pollen (25 to 53.9%) from related species than the tetraploid *S. scabrum* (2n = 48; 0 to 11%). We concluded that insect-pollinators complement self pollination in *Solanum*. They provide cross-pollen, which enhanced gene exchange and hybrids in natural population and lower inbreeding depression.

Key words: Breeding system, hybridization, insect pollinator, outcrossing, pollination, *Solanum*.

INTRODUCTION

Plant-pollinator interactions though extremely variable, is one of the most common mutualism in nature through which plants offer rewards to flower visitors and they inadvertently transfer pollen among conspecific plants to effect fertilization (Waser, 1983; Sahli and Conner, 2007). Variation in floral morphology and characteristics is core to our understanding of the processes and patterns of breeding systems, seed production, and the degree of mutual interdependence between a species and its biotic community (Galen and Cuba, 2001; Waites and Agren, 2006; Smith-Ra mirez et al., 2005). Floral architecture may reproductively isolate (conserve) a species or engender a range of possible evolutionary outcomes. It determines the extent of hybridization and integration of foreign genes, necessary for species adaptation and survival. Overall, floral morphology, display, colour, flowering phenology and quality of nectar and pollen determine the number and diversity of insect pollinators (Devy and Davidar, 2003).

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Although, many species of *Solanum* are self compatible, a great number requires outcrossing for greater fruit set (Amoako and Yeboah-Gyan, 1991; Kowalska, 2008). Prolonged selfing may result in the expression of deleterious recessive alleles leading to inbreeding depression (Charlesworth and Charlesworth, 1987; Barret and Harder, 1996; Johnston, 1998). Evolution of mixed mating system may lower inbreeding depression in angiosperm. About one third of flowering plants maintained a mixed mating system involving both self and outcrossing (Barrett et al., 1996; Vogler and Kalisz, 2001).

Insect pollinators play effective role in gene flow within intra- and inter-specific populations engendering species diversity and population heterogeneity, and inevitably, evolutionary changes over time. Impairment of plant-pollinator interaction may lead to species population fragmentation and extinction. Therefore, the foraging pattern of pollinators influences plant reproductive success, mating systems and spatial structure of species population (Harder and Barrett, 1996; Barrett, 2003; Mitchell et al., 2005). Nectar foraging insects have been found to be more efficient than pollen-seeking insects (Wilson and Thomson, 1991).

In sexual hermaphroditic plants, low fruit-to-flower ratio may result from predation (Ugborogho and Oyelana, 1993, 1999), insufficient pollen contact or lack of resources to attract pollinators (Johnston, 1991; Vesprini and Galetto, 2000). Single pollinator probes are sometimes insufficient for maximal seed set, hence, multiple probes may suffice. The number of probes to individual flowers in self-compatible species influences the proportion, quality and genetic diversity of outcrossed progeny and the nature of paternity within fruits (Mitchell et al., 2005; Karron et al., 2006).

The relative abundance, effectiveness and visitation rate of each pollinator-taxon vary temporally and spatially with great impact on pollination efficiency, patterns of plant reproduction and floral evolution (Fishbein and Venable, 1996; Ivey et al., 2003). Environmental fluctuations causing changes in population dynamics of pollinator species may also result in temporal variation in pollinator assemblages (Herrera, 1988). Such fluctuations in the frequency of visitation can alter selective pressure, specialization and adaptation to floral traits by any pollinator (Schemske and Horvitz, 1989; Pettersson, 1991). Pollination effectiveness has been quantified in terms of the amount of removal and or deposition of pollen (Ivey et al., 2003), pollen load on pollinators (Moeller, 2005), probability of contacting stigmas and anthers (Armbruster, 1988) and sometimes seed set (Wiggam and Ferguson, 2005). Plants have therefore, evolved a suite of characteristics that enable them to exploit the morphology, sensory mechanisms, needs, and overall behaviour of their animal visitors (Mayfield et al., 2001). Despite the potentials for mutual benefits, plant-pollinator interactions could also be in the form of reciprocal exploitation in which animals forage for floral rewards without compensating their host plants with benefits (Maloof and Inouye, 2000). Pollination effectiveness thus considers the total contribution by pollinators to plant fitness.

This paper assesses the extent of plant-pollinator interaction in *Solanum* based on floral morphology and breeding systems and the effects on pollination efficiency, fruit and seed set.

MATERIALS AND METHODS

Study species

We differentiated nine taxa for our study. These included two complex species (*Solanum melongena* and *Solanum scabrum*) of four taxa. *S. melongena* L. *'Melogena'* possessed purple fruit when ripe, while *S. melongena* 'Golden' had yellow fruit. *S. scabrum* Mill. (*Solanum nigrum* L. var. *guineensis*; indigenous name (Yoruba): Odu) is believed to have originated from West and Central Africa because of its diversity in Nigeria and Cameroon (Gruben and Denton, 2004). Our *S. scabrum* 'Scabrum' has procumbent rectangular stem with 11.4 x 10.8 cm entire leaves, petals 5.9 x 3.6 mm and fruits 7.5 mm diameter. *S. scabrum* 'Erectum' on the other hand has erect round stem with 6.6 x 3.5 cm serrated-margin leaves, petals 3.5 x 1.5 mm and fruits 5.1 mm diameter.

Other species were: *S. aethiopicum* L., *S. anguivi* Lam. (*Solanum indicum* L. subsp. *distichum* Schum. & Thonn.) Bitter, *Solanum gilo* Raddi, *Solanum erianthum* Don and *Solanum torvum* SW. *Solanum anguivi* is native to Africa, grows mostly in the wild, but sometimes a semi-cultivated vegetable in Uganda and Côte d'Ivoire. The species are autogamous, with a strong tendency to outcross when insect pollinators are available.

Study sites

The study was initially conducted at the Biological garden of the University of Lagos, Lagos. We confirmed our earlier observations and recovered our plant pollinators in rural Babcock University, Ilishan-Remo, Biological Garden, 75 km away from our initial urban study site. In both cases, study species were grown form seeds on soil beds. The perennial, *S. erianthum* was studied in its natural habitat at Ijebu-Ode, Ogun state, 110 km from University of Lagos and 35 km from Babcock University.

Thirty-six planting beds measuring 6 x 4.5 m with 0.5 m spacing along and between rows on a 60 x 30 m plot were utilized. The beds were mulched for four weeks prior to receiving seedlings. The seedlings were shaded to nurse for more than three weeks. The young seedlings were transferred to the field in the early hours (06:00 to 08:00 h) in open-bottom planting bags. They were placed in 25 cm holes and spaced 1.3 m apart, along and between rows. Each bed accommodated 12 plants, which were watered twice daily, at early hours and sunset.

Analysis of floral features

We examined placentation, floral whorls and parts including the petals, sepals, pistils and stamens with the aid of a hand lens and a stereomicroscope (Wild). The lengths and breadths of petals, sepals, stamens, pistils, pedicels and the diameters of flowers and mature fruits were measured with a meter rule and the data was subjected to analysis of variance (ANOVA).

Reproductive biology

Stigma receptivity

Forty-eight randomly selected mature flower buds on 12 different plants were emasculated per taxon 12 h prior to opening of petals, bagged and tagged A1, A2 to X1, X2. Each stigma was rubbed with pollen from anthers of neighbouring flowers from the same plant at intervals of 10 min, 1 h before petals opened. The last application (X – stigma) received pollen about 4 h after the first application. At each interval, two flowers on two different plants were considered per species. The treated flowers were observed for fruit set to determine when stigmas became receptive to compatible pollen.

Pollen viability, pollen load and flower count

Pollen from anthers soaked overnight in 90% alcohol were teased out on glass slides, mounted directly in 1:1 glyceroacetocarmine and kept in an oven at 60 °C for 24 h. The pollen viability was determined from the degree of stainability of pollen with full cytoplasm. The diameters of pollen were measured with an eyepiece graticule at 400x on an Mka 5 Wild Compound Light Microscope. We estimated pollen load per flower from the total sum of pollen per anther in a roll of stamen. We obtained an average from 25 randomly selected flowers per species on five different days and multiplied them with the average number of flowers opened per plant per day for plant load per species. Five randomly selected plants were tagged and observed per day for five different days per species. Overall, 1762 flowers on 225 plants were observed for the nine taxa studied. Growth of flowers was monitored from bud initiation to setting of fruits. Counting was discontinued at the setting of fruits on each inflorescence when the stamens and corolla were shed.

Anther dehiscence

Thirty-six randomly selected mature flower buds on 12 different plants were bagged per species. Anthers were checked for pollen drops at intervals of 10 min, 1 h before petals opened. At each interval, two flowers on two different plants were considered per species. The tip of each bud was lifted open for a visual observation of the anthers. The detailed examination of the anther's pore was carried out with the help of a hand lens. Bags were removed at the end of the observation.

Insect visitors

Pollinator watch was conducted at regular intervals for the entire growing season. Periodic daily observation commenced before the opening of flowers and lasted till flowers were fully closed. The techniques of Ugborogho and Oyelana (1993) were employed to determine the status of insect visitors and their pattern of pollen transfer across the different flowers. Some of the insect visitors were trapped, marked and later released. Their activities were monitored daily to determine the frequency and duration on different flowers. At intervals, some of the marked insects were caught and examined for pollen attachment. The regions of integument utilized to determine pollen distribution on the bodies of these insects included the antennae, mouth parts such as the mandible, maxillae, labrum and proboscis. Other parts included dorsal and ventral cervix, wings and segments of the abdomen and legs.

Pollination efficiency and fruit set

We measured pollination efficiency from the number of fruit set from pollinated flowers. We also assessed the number of flowers pollinated from the total number of opened flowers under natural conditions. Pollinated flowers were observed for fruit development. The days taken by a fruit to ripe was calculated from the shedding of corolla to ripening. The length of fruiting was determined by selecting five plants per species and estimating the number of days between the first and the last fruit on a number of inflorescence. The average was scored for each species. The average number of seeds per fruit was also determined from a total of 35 randomly selected fruits per taxon.

Breeding systems

For each species, 6 to 16 mature flower buds were tagged and bagged 12 h prior to opening and categorized into five groups:

1. Open (control): Unbagged flowers allowed to self-pollinate under natural conditions.
2. Bagged (pollinator-exclusion): Bagged flowers allowed to self-pollinate in the open.
3. Selfed: Bagged flowers hand pollinated with own pollen, pollen from flowers on the same or other inflorescence from the same species.
4. Crossed: Flowers emasculated, pollinated with pollen from another species and bagged.
5. Emasculated: Flowers emasculated, bagged and left unpollinated.

We used the first three treatments to assess flowers for self-compatibility. The fourth treatment assessed the potential for hybridization, while the fifth assessed flowers for obligate agamospermy. The fruits from the different treatments were counted, measured and number of seeds estimated. For cross-pollination experiment, the following crosses were undertaken:

1. *S. melongena* 'Golden' (2n = 24) x *S. melongena* 'Melongena' (2n = 24)
2. *S. aethiopicum* (2n = 24) x *S. melongena* 'Golden' (2n = 24)
3. *S. torvum* (2n = 24) x *S. aethiopicum* (2n = 24)
4. *S. anguivi* (2n = 24) x *S. torvum* (2n = 24)
5. *S. melongena* 'Golden' (2n = 24) x *S. anguivi* (2n = 24)
6. *S. erianthum* (2n = 24) x *S. torvum* (2n = 24)
7. *S. aethiopicum* (2n = 24) x *S. scabrum* 'Scabrum' (2n = 48)
8. *S. torvum* (2n = 24) x *S. scabrum* 'Scabrum' (2n = 48)
9. *S. anguivi* (2n = 24) x *S. scabrum* 'Erectum' (2n = 48)
10. *S. melongena* 'Melongena' (2n = 24) x *S. scabrum* 'Erectum' (2n = 48).

RESULTS

Inflorescence

Inflorescence was compound dichasium (*S. erianthum* Don and *S. torvum* SW. (Figure 1A and B) or simple (Figure 1C and D) as in the other species. The compound dichasium types were often terminal on main and lateral shoots, while the simple inflorescences were basically lateral, attached at any point along the axis of stems and branches. The latter was found in *S. aethiopicum* L., *S. scabrum* Mill. 'Scabrum' and 'Erectum', *S. gilo* Raddi, and *S. melongena* L. 'Melongena' and Golden'. In compound

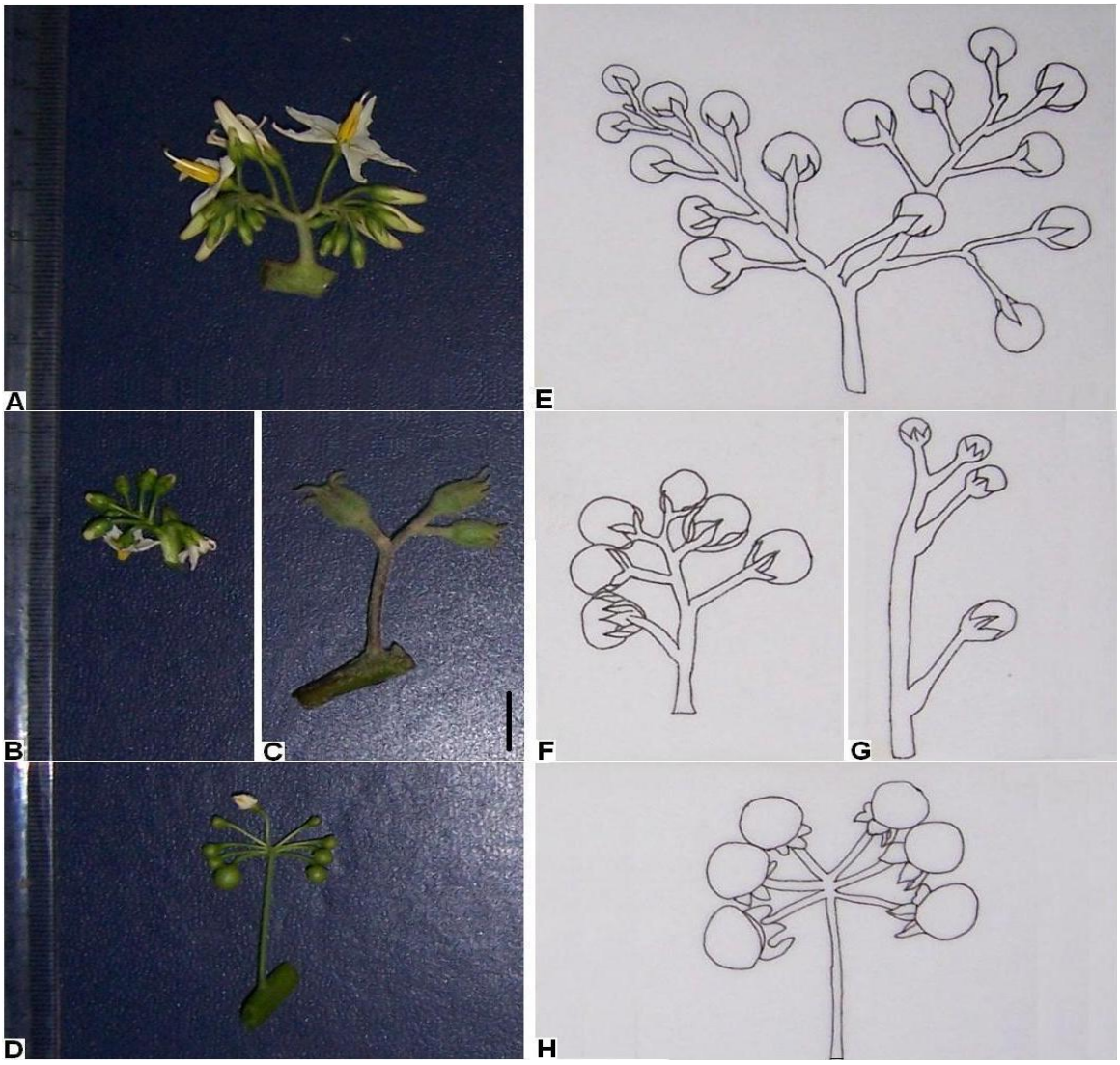

Figure 1. Types of inflorescences in *Solanum*. A. Compound dichasium; B. Compound monochassium; C. Raceme; D. Umbellate; E to H: Illustrative drawing of A to D, respectively. Scale bar: A to D = 10 mm.

dichasium, branching of peduncle may be either monochasial (*S. anguivi* Lam.) (Figure 1B and F) or dichasial (*S. erianthum* and *S. torvum*) (Figure 1A and E), having eight to 12 sympodial flowers.

The simple inflorescence type was either extra-axillary (*S. aethiopicum)* having five to six sympodial flowers or inserted directly on main axis or branches with umbellate flowers *(S. scabrum* complex) (Figure 1D and H) or raceme (*S. gilo* and *S. melongena* complex) (Figure 1C and G). The few solitary flowers seen were inserted directly on stems or branches of *S. gilo* and *S. melongena.*

Anthesis

In all the species except *S. erianthum*, flower buds emerged eight weeks from planting and the plants flowered throughout the entire growing season. *S. erianthum* is a perennial plant whose flowering commenced in the middle of the second growing season (18 months) till the end of the study. The flowering periods greatly overlapped and the species blossomed till the end of the planting season. The flower buds opened between 2 and $2^1/_2$ weeks from initiation. Opening was asynchronous and dependent on age and health of the buds.

Flowers commenced opening between 05:40 and 06:20 h in *S. anguivi*, *S. aethiopicum* and the *S. scabrum* complex. In *S. gilo* and *S. melongena* complex, opening was between 06:40 and 07:50 h, while flowers opened from 06:55 to 07:35 h in *S. torvum* and *S. erianthum*. The flowers opened fully within 20 and 25 min from initiation in all the species. At the commencement of opening of

flowers in *S. scabrum* 'Scabrum', one or two petals slide opened and the other petals opened one after the other in a sequence. The loosed corolla eventually spread out, looped backwards and their tips hinged on the receptacle.

In *S. melongena* 'Golden', opening followed a different procedure. The corolla first became bulbous, then attained a characteristic cone shape and subsequently split along the seam with each petal spreading backwards independently. The duration of opening was between 10 and 13 h, and petals closed up afterwards. The closing procedure in both species was identical and the mechanism followed the reverse sequence of the opening process. Each petal folded inwards, starting with the last petal to open, and corolla eventually formed a loose envelope around the pistil. Flowers attained full closure within 25 and 35 min.

Floral structure

The flowers were at obtuse angle in relation to the pedicel. Petals were five and pink-purple in *S. gilo* and *S. melongena*, but white in the other species (Figure 2A to D). In all the species, aestivation was valvate. Sepals were five, lobed and foliaceous. Stamens were five and equal. Anthers were oblong and bilobed. Styles were glabrous, exersted and terminate with bilobate stigmas but hairy in *S. scabrum* complex. Ovary was glabrous with numerous ovules.

Reproductive biology

Stigma receptivity

Stigma became receptive about 45 min before anthesis in *S. aethiopicum, S. gilo, S. anguivi* and *S. scabrum* complex. Receptivity was about 60 min before flowers opened in *S. torvum*, *S. erianthum* and *S. melongena*.

Pollen

Pollen may be monoporate-round, diporate-oblong, triporate-triangular or tetraporate-rectangular in type and shape. The largest pollen, tetraporate, was found only in *S. gilo.* The size distribution ranged from 23.5 to 50 µm. The average size distribution ranged from 27.8 to 36.8 µm. Fewer pollen were found below 26.8 µm and above 40.1 µm size ranges (Figure 3A and B)

The number of viable pollen ranged from 1912 in *S. melongena* 'Golden' to 5208 in *S. torvum.* This represents 54.8 and 97.8% of flower (pollen) load, respectively. Pollen load varied between 3167 in *S. melongena* 'Melongena' and 5837 in *S. scabrum* 'Erectum' per flower (Figure 4 A). While pollen load was close among the closely related taxa of *S. melongena* (3274 and 3167) and *S. scabrum*, their pollen viability (58.4 and 71%) varied considerably. Anther and pollen sizes were negatively correlated with pollen load, except in *S. torvum*. Short anthers (1.5 to 2.5 mm) and small pollen (27.8 to 32.2 µm) in *S. scabrum* complex and *S. erianthum* ensured large pollen load per flower (4129 to 5837) and relatively high pollination efficiency (79.6) (93.3 to 97.7%). Conversely, long anthers (5 to 8.5mm) and large pollen (36.1 to 41.9 µm) in *S. melongena* complex and *S. gilo* resulted in lower pollen load (3167 to 3874) and reduced pollination efficiency (73.9 to 80%) (88.4). *S. aethiopicum* and *S. anguivi* were intermediate in anther (3 to 5 mm) and pollen sizes (31.1 to 36.2 µm) with relatively moderate to high load (4996 to 5173) and pollination efficiency (82.9 to 96.6%) (Figure 4A and C). Pollen load per plant (140,782 to 2,098,050) (6,070,483) was largely dependent on pollen sizes (Figure 4). Table 1 shows the mean sizes of floral parts for the different species and pollen load per plant.

Anther dehiscence

Anther's dehiscence was diurnal and occurred ca. 20 min after the opening of flowers in *S. torvum*, *S. anguivi* and *S. erianthum*, while it took place ca. 30 min prior to the opening of flowers in *S. gilo*, *S. melongena* complex and *S. scabrum* complex. Each anther dropped its pollen intermittently, spanning the entire duration of opening of flower in all the species. The peak of pollen release took place between 20 and 30 min of anther's dehiscence. Anther's dehiscence was synchronous in several flowers, especially in those of the same inflorescence.

Pollination

Pollination could be achieved anytime from anthesis to shortly before the closing of flowers in all the species. Pollinated flowers shed their corolla from the third day pollination was effected. Unpollinated flowers reopened the next day and eventually dropped if pollination fails.

Pollination efficiency (73.9 to 98.2%) and fruit-set (66.1 to 91.1%) from open pollinated flowers were generally high, except in *S. melongena* complex and *S. gilo* where floral predation, failure of fertilisation or abortion of fertilised ovule affected fruit set (35 to 39.1%) considerably (Figure 4B and C). Anther and pollen sizes and hence pollen load influenced pollination efficiency and also outcrossing by insect pollinators. Thus, in spite of low pollen viability in *S. melongena* 'Golden' (58.4%), pollination efficiency (88.4%) was higher than its close relative, *S. melongena* 'Melongena' (73.9%) with considerably higher pollen viability (71%). Similarly, in *S. gilo* and *S. erianthum* (Figure 4C). Pollination was difficult in flowers that did not form the peculiar obtuse angle curvature on the inflorescence axis. Efficiency with respect to the number of flowers openly pollinated

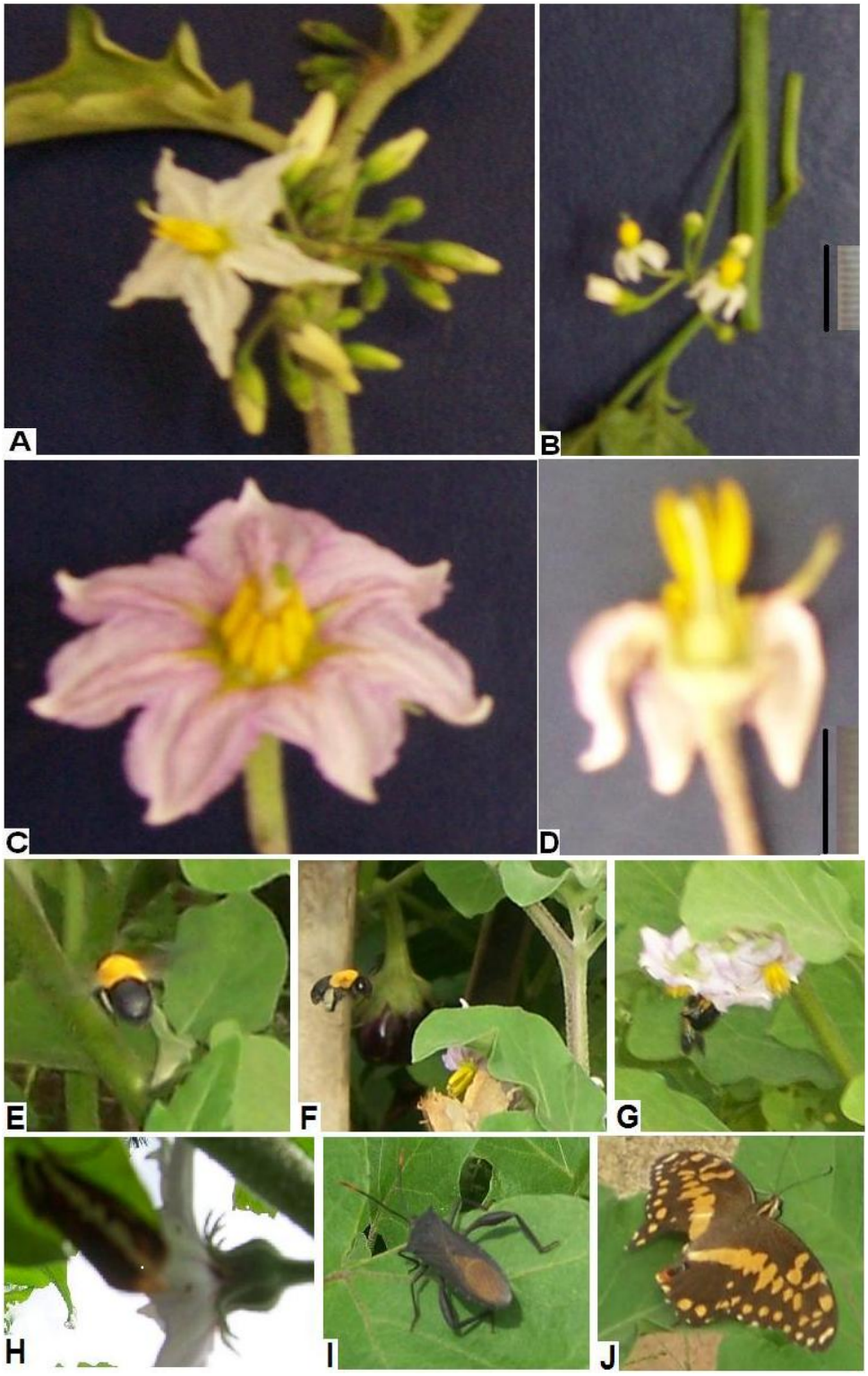

Figure 2. Floral structure, insect pollinators and visitors to flowers of *Solanum*. A to C. Fully open flowers: A. *S. torvum*; B. *S. aethiopicum*; C. *S. melongena* 'Melongena'; D. LS of flower of *S. melongena* 'Melongena'; E to G. Activities of insect pollinator of *S. melongena 'Melongena'*–*Bombus pennsylvanicus.* E. Prospecting an open flower; F. Set on target flower; G. Alighted on flower and sucked pollen; H to J. Insect visitors and ovipositor on *S. melongena* 'Melongena'; H. Moth taking shelter on leaf; I. *Anasa tritis*- ovipositor; J. *Papilio cresphontes*, a casual visitor, resting on leaf. Scale bar: A to C, D = 10 mm.

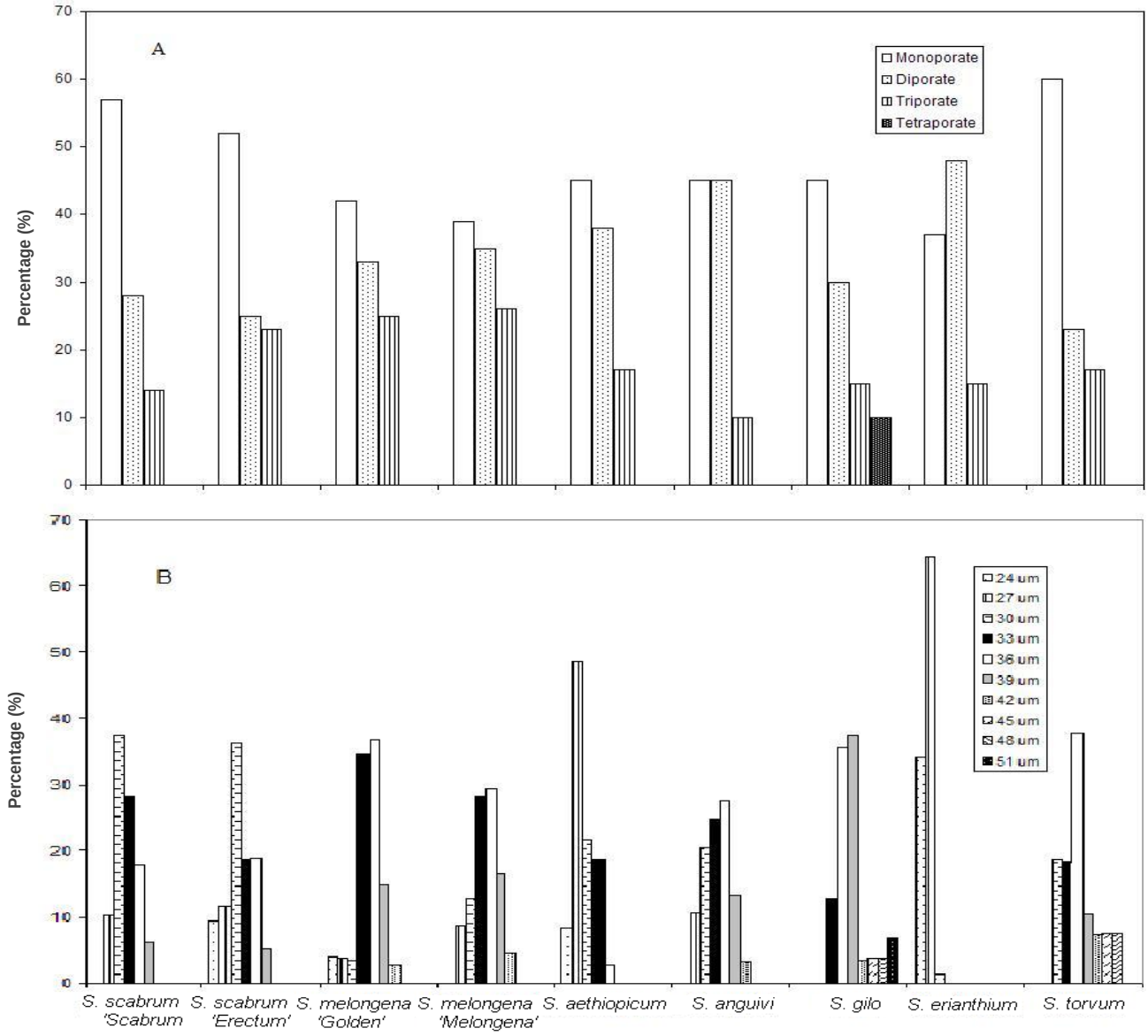

Figure 3. Frequency distribution of pollen types.

was consistent with the established anther-pollen size and load pattern. It was low to average (41 to 52.9%) in the large anther/pollen and small pollen load taxa (*S. melogena* complex and *S. gilo*) and high (83.2 to 90.1%) in the small anther/pollen and large pollen load taxa (*S. scabrum* complex and *S. erianthum*). *S. anguivi* and *S. aethiopicum* were intermediate (68.5 to 83.1%), while *S. torvum* was anomalous with large anther/pollen, high pollen load and highest pollination efficiency (92.7%) (Figure 4A).

Fruit development

Fertilized ovary took 3½ to 4 weeks to mature into fruits in *S. aethiopicum, S. anguivi, S. torvum,S. erianthum* and *S. scabrum complex* and 5 to 6 weeks in *S. gilo* and *S. melongena* complex. The number of seeds per fruit varied, even among fruits borne on same inflorescence. An average of 58 and 384 seeds per fruit was scored, respectively for *S. anguivi* and *S. melongena* 'Golden'. The quantity of seed-set is dependent upon the size of the fruit particularly, the length (Figure 4 D).

Fruits varied in shape and colour. It was round and green in *S. torvum*, globose in *S. melongena*, yellow in 'Golden' and purple in 'Melongena'. It was oblong and yellow in *S. gilo* and generally round in the other species, red when ripe in *S. aethiopicum* and *S. anguivi*, yellow in *S. erianthum* and *S. torvum*, and purple in *S. scabrum* complex. Seeds were brown and ovoid-reniform in *S. gilo*

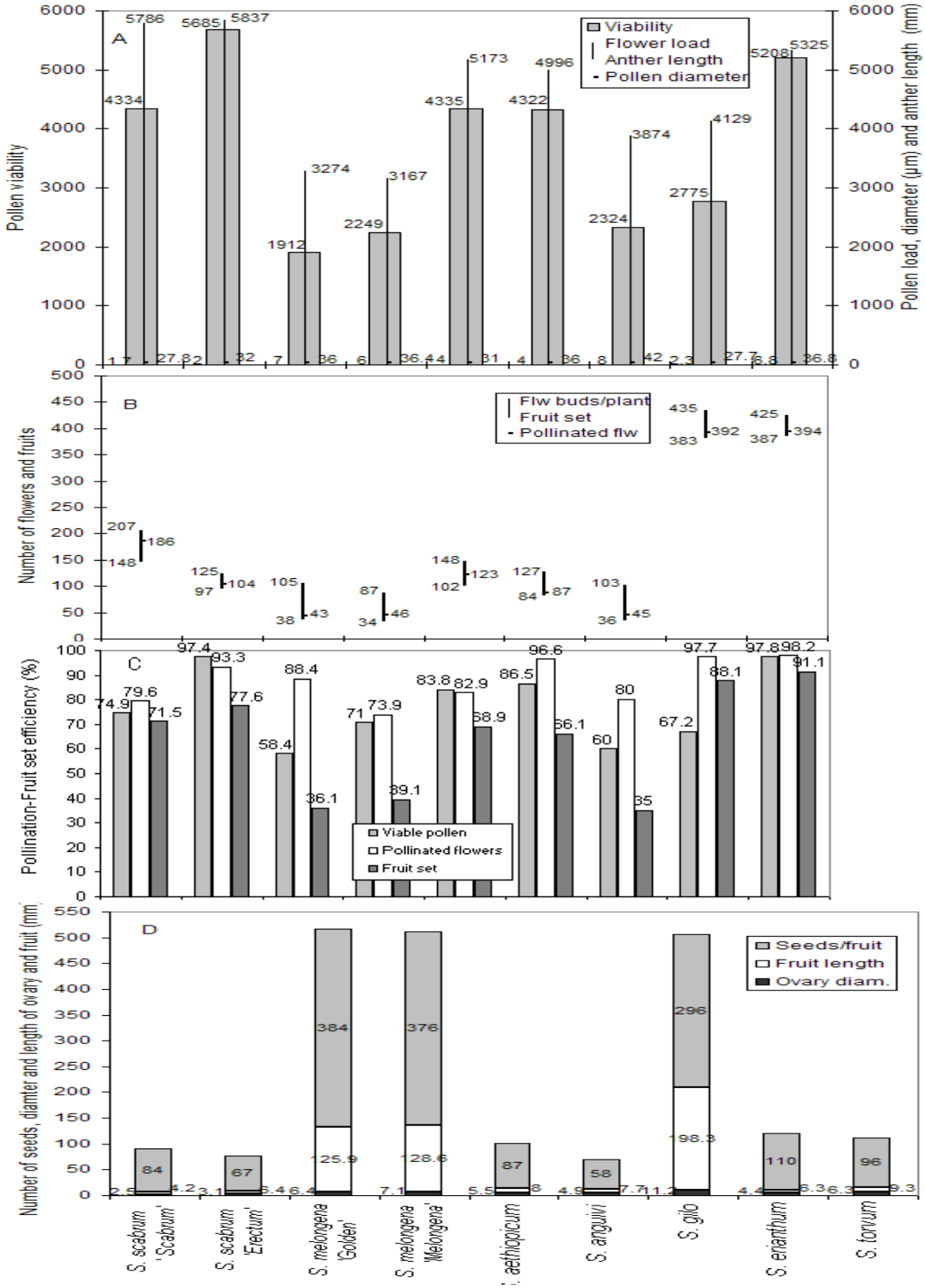

Figure 4. Reproductive biology in *Solanum*. A. Pollen load, sizes and viability; Column = number of viable pollen; Line: upper limit = pollen load per flower; point along the line = diameter of pollen (27.7 to 41.9 µm); lower limit = length of anther (8 to 17 mm). B. Fruit set. Line; Upper limit = number of flower buds per plant; point along the line = number of flowers pollinated; lower limit = number of fruit set; C. Pollination efficiency and fruit set; D. Number and sizes of seeds, ovary and fruit.

Table 1. Floral characters of *Solanum* species.

Species	Number of flowers measured	Petal length (mm)	Petal breadth (mm)	Diameter of open flower (mm)	Sepal length (mm)	Sepal breadth (mm)	Anther length (mm)	[§]Pollen load/ plant	[§]Seeds Average/ Plant	Style length (mm)	Pistil length mm)	Pedicel length (mm)
*S. *scabrum* 'Scabrum'	70	3 – 4 [†]3.5±0.5	1 – 1.8 1.5±0.3	4.3±0.5	1 – 2 1.75±0.4	0.8 – 1 1.0±0.1	1.5 – 2 1.7±0.2	1,076,196	12,432	2 – 3 2.6±0.5	3.5 - 4 3.8±0.3	5 – 6 5.5±0.5
*S. *scabrum* 'Erectum'	70	5 – 7 5.9±0.7	3 – 5 3.6±0.9	5.5±0.5	2.5 – 3 2.6±0.2	1.5 – 2 1.9±0.2	1.5 – 2.2 1.95±0.2	6,070,483	6,499	3 – 4 3.5±0.4	5 – 6 5.4±0.3	10 - 12 10.4±0.8
S. melongena 'Golden'	40	17 – 19 17.8±0.8	5 – 7 5.8±0.8	33.4±1.2	8 – 10 9.4±0.9	3 – 4 3.2±0.5	7 – 8 7.1±0.4	140,782	14,592	8 – 11 10.4±0.6	11 - 13 12.2±0.4	*15 - 33 26.2±3.1
S. melongena 'Melongena'	40	15 – 20 18.4±1.5	5 – 8 6.8±1	34.1±1.5	8 - 11 10±0.9	2 – 4 3.2±0.7	5 – 8 6±1.2	145,682	12,784	9 – 10 9.7±0.6	10 - 16 15.5±0.7	*34 – 40 37.1±0.4
S. aethiopicum	50	7 – 9 7.1±0.9	3 – 5 3.9±0.7	8.2±0.9	2 – 3 2.8±0.4	1.5 - 2.5 2±0.3	4 – 5 4.1±0.4	636,279	8,874	4 – 5 4.3±0.6	6 – 7 6.7±0.6	4 – 8 6.3±2.1
S. anguivi	50	5 – 8 6.3±1	3 – 4 3.9±0.3	8±0.7	3 – 4 3.6±0.5	2 – 3 2.1±0.3	3 – 4 3.9±0.3	434,652	11,426	4 – 5 4.5±0.6	3.5 – 4 3.85±0.3	6 – 8 7±1.2
S. gilo	40	9 – 12 10±1.1	3 – 4 3.5±0.6	38.6±1.9	4 – 7 5.8±1.3	3 – 4 3.3±0.5	7 – 8.5 7.8±0.55	174,330	10,656	7.5 – 10 9.8±0.5	11 – 13 12.5±0.4	15 – 28 25.4±3.2
S. erianthum	60	6 – 7 6.8±0.5	2.5 – 3 2.9±0.2	7.8±0.5	3.5 – 5 4.1±0.4	2 – 3 2.6±0.4	2 – 2.5 2.3±0.3	1,618,568	42,130	4 – 6 5±0.8	7 – 8 7.7±0.6	5 – 10 6.9±2
S. torvum	60	12 – 13 12.8±0.42	5 – 6 5.2±0.4	21.4±1.4	3 – 5 4.1±0.7	1 – 2 1.7±0.4	6 – 7 6.8±0.4	2,098,050	37,152	6 – 10.5 9.4±1.7	6 – 13 12±1.3	10–13 10.8±1.3

[§]Number of flowers and fruits considered per plant are indicated in Figure 4B. Significant *P ≤ 0.05 for floral dimensions except anther's length for S. scabrum; [†]Mean ± SD.

and *S. melongena* but were yellow and flattened-reniform in the other species (Figure 5).

Insect visitors and pollinators

The insect pollinators included *Megachille latimus*, *Diplolepis rosae*, Bumblebee–*Bombus pennsylavanicus* (Hymenoptera) and the nymphs of *Gryllus pennylavanicus* (Orthoptera). Visits began from 06:30 h and reached the peak at full anthesis in all the species. This coincided with the peak of pollen shedding in *S. torvum* and *S. erianthum. M. latimanus, D. rosae* and *B. pennsylvanicus* are flying insects which made quick and repeated visits to several flowers. Each visit was brief, usually less than 10 s. They alighted directly on the stamens and sucked pollen from dehisced anthers. Consequently, they rubbed their ventral and abdominal segments on the exersted stigmatic surfaces repeatedly (Figure

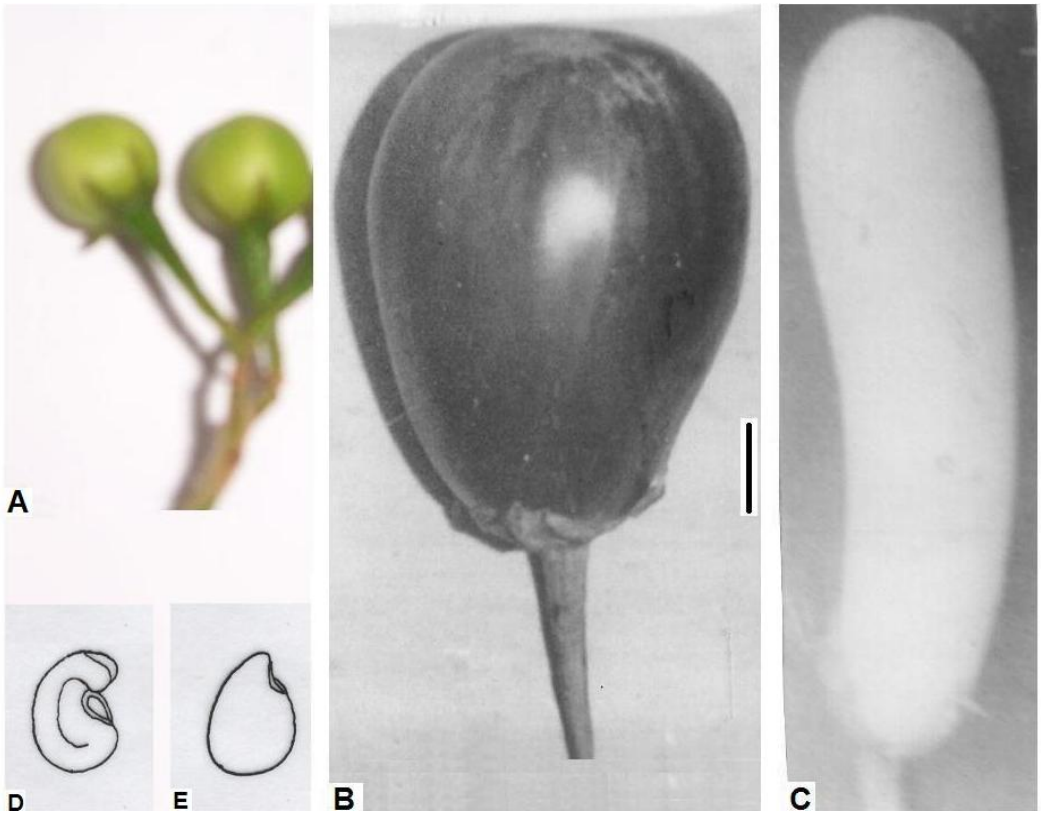

Figure 5. Fruits of *Solanum*. A. *S. torvum*, round; B. *S. melongena* 'Melongena', globose; C. *S. gilo,* oblong. D and E. Seed. D. Ovoid-reniform; E. Flattened-reniform; Scale bar: A to C = 10 mm, D and E = 2 mm.

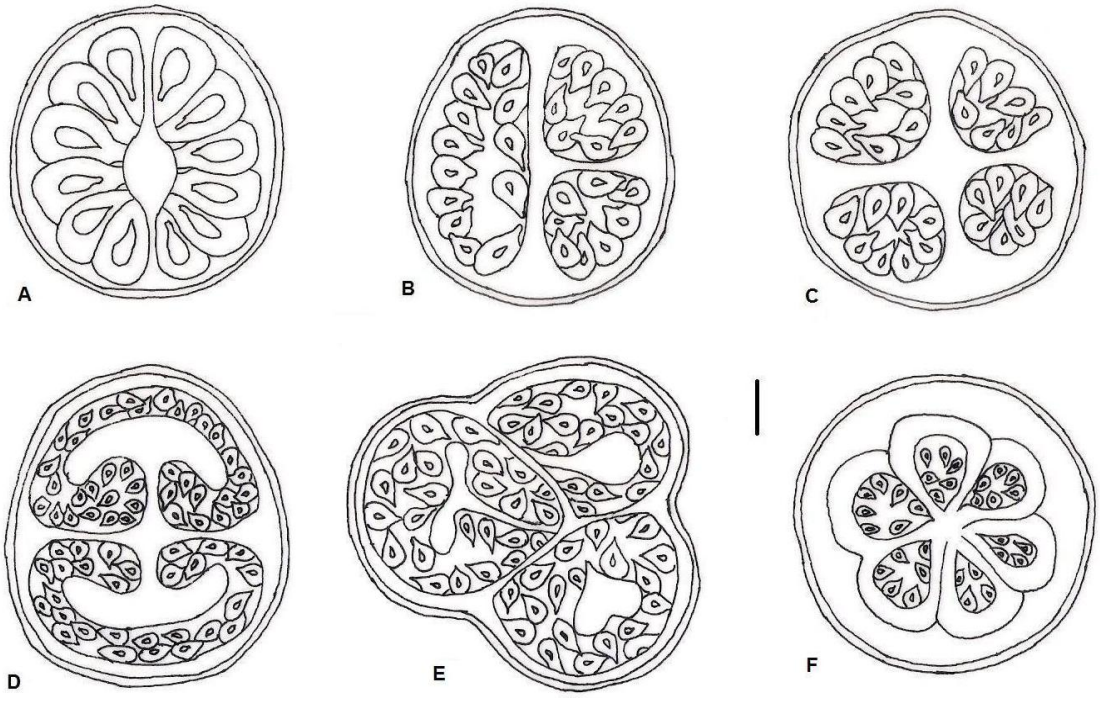

Figure 6. Placentation types. A. Two-carpel; B. Three-carpel; C and D. Four-carpel. E and F. Six-carpel. Scale bar = 1 mm.

Table 2. Fruit and seed set from open and controlled pollination of *Solanum* species.

Species	Open					Bagged					Selfed					Crossed					Emasculated				
	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e
S. scabrum 'Scabrum'	5	4	80	82	5.0	4	2	50	37	3.8	5	5	100	97	5.9	8	1	12.5	15	6.2	10	-	-	-	-
S. scabrum 'Erectum'	7	5	71.4	73	5.9	3	2	66.7	44	5.4	6	6	100	94	7.2	8	0	0	0	0	12	-	-	-	-
S. melongena 'Golden'	4	4	100	66	131.7	3	3	100	38	124.0	4	4	100	89	138.7	4	1	25	12	97.2	8	-	-	-	-
S. melongena 'Melongena'	5	4	80	79	122.7	4	3	75	41	118.3	4	4	100	102	131.3	4	3	75	48	87.3	8	-	-	-	-
S.aethiopicum	4	3	75	67	7.6	4	2	50	34	6.7	6	6	100	93	8.3	5	3	60	36	5.0	6	-	-	-	-
S. anguivi	8	6	75	64	6.9	5	3	60	47	6.3	5	5	100	87	7.0	6	4	66.7	33	4.8	10	-	-	-	-
S. gilo	8	7	87.5	148	168.8	3	2	66.7	89	143.7	4	4	100	164	177.8	5	3	60	78	110.6	8	-	-	-	-
S. erianthum	5	4	80	94	6.2	5	3	60	56	6.0	4	4	100	112	6.9	4	2	50	21	5.1	10	-	-	-	-
S. torvum	5	3	60	72	9.4	4	2	50	45	6.6	5	5	100	88	9.9	4	2	50	28	5.7	12	-	-	-	-

a: Number of flowers pollinated, b: number of fruit set, c: percentage pollination efficiency, d: average number of seeds per fruit, e: mean diameter of fruits (mm).

2E to G). Usually, they visited dozens of flowers in less than 10 min in the early hours of morning as anthesis commenced. Pollen was observed on the legs, mouthparts and ventral parts of thorax of the insects. These insects provided effective means of pollen transfer among flowers.

The nymphs of *G. pennylvanicus* were light hoppers and were less mobile. They encountered few flowers and often hopped from one petal to the other in order to pick few pollen that dropped off the anthers. They rarely made contacts with the stamens and stigmas. Pollen was observed mostly on their abdominal segments.

The third group of insect visitors were the Moth, squash bug– *Anasa tristis* (Hemiptera) and swallowtail butterfly– *Papilio cresphontes* (Lepidoptera) (Figure 2H to J). *A. tristis* oviposited on young flower buds. They were usually prevalent on the flowers of *S. gilo* and *S. melongena.* They practically domiciled on these plants, and burrowed into emerging flower buds to oviposit. Emerging larvae fed on the pollen and destroyed the stamens in the process. The affected flowers did not open but eventually dropped. Over 60% of the total number of flowers was destroyed by the activities of these insects. *P. cresphontes* and moth were occasional visitors taking brief or long rest on the plants.

Placentation

All the species were characterised by superior ovary. The ovary was made up of two to six carpels, each consisting of numerous ovules. The placentation type is axile and the different types are described in Figure 6. The growth and characteristic shape of the septa divided the ovary into carpels. An ovary may consist of two carpels (*S. erianthum* and *S. torvum*) (Figure 6A and B), four carpels (*S. aethiopicum* and *S. anguivi*) (Figure 6C and D) or five to six carpels (*S. scabrum* complex) (Figure 6E and F).

Breeding systems

Maximum pollination was achieved from hand-pollinated flowers (selfed) with 100% fruit set in all the species. Pollination efficiency decreased slightly in the open (60 to 87.5%) under natural conditions and reduced further in open bagged flowers (50 to 75%) and outcrossed pollen (0 to 75%). Pollination efficiency and fruit set were maximal (100%) for *S. melongena* 'Golden' under the three conditions, selfed, open and bagged (Table 2). *S. scabrum* 'Erectum' did not receive own pollen, while its relative, *S. scabrum* 'Scabrum' barely received only 12.5% of own pollen from other flowers.

Hybridization success was average in crosses involving *S. melongena*, *S. anguivi* (50 to 53.9 %) and below average in *S. aethiopicum*, *S. torvum* and *S. erianthum* (25 to 41.7 %), all diploids (2n = 24) (Figure 7 A). Crosses involving *S. scabrum* (a tetraploid, 2n = 48) (Figure 7 B) were either unsuccessful or insignificantly low (3.8 to 11.1%) (Table 3).

The fruits from hand-pollinated flowers were relatively bigger with more seeds than the bagged and open flowers (Table 3). The number of fruits and seeds were fewer in the hybridized plants. All the emasculated flowers did not set fruit, showing no evidence of obligate apomixes in all the species.

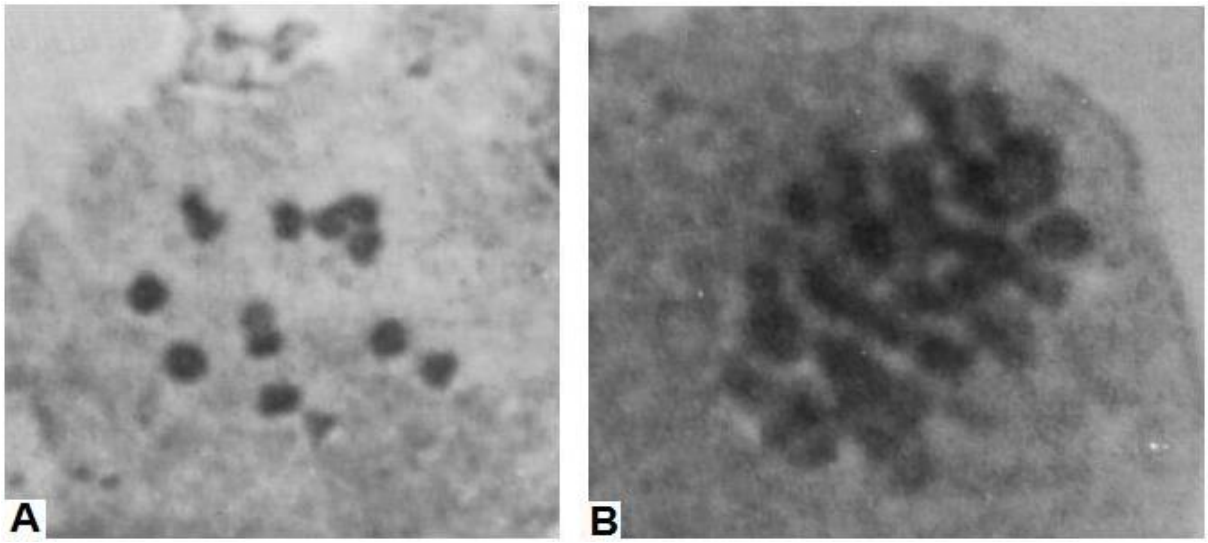

Figure 7. Meiotic chromosomes of *Solanum*. A. *S. gilo,* n = 12; B. *S. scabrum* 'Erectum', n = 24.

Table 3. Fruit set and number of seeds from cross-pollination experiments in *Solanum.*

Female parent	F_1 Character					Male parent
	a	b	c	d	e	
S. melongena 'Golden'	35	31	88.6	28	97.2	*S. melongena*' Melongena'
S. aethiopicum	24	10	41.7	17	5.0	*S. melongena* 'Golden'
S. torvum	34	13	38.2	22	6.7	*S. aethiopicum*
S. anguivi	22	11	50	24	5.0	*S. torvum*
S. melongena 'Golden'	26	14	53.9	36	67.3	*S. anguivi*
S. erianthum	32	08	25	16	5.1	*S. torvum*
S. aethiopicum	35	02	5.7	08	6.2	*S. scabrum* 'Scabrum'
S. torvum	24	0	0	-	-	*S. scabrum* 'Scabrum'
S. anguivi	26	01	3.8	12	13.4	*S. scabrum* 'Erectum'
S. melongena 'Melongena'	18	02	11.1	16	10.2	*S. scabrum* 'Erectum'

a: Number of flowers pollinated, b: number of fruit set, c: percentage pollination efficiency, d: average number of seeds per fruit, e: mean diameter of fruits (mm).

Their flowers reopened for an additional day and subsequently dropped.

DISCUSSION

The breeding systems of most angiosperm families are controlled by one or combination of pollination conditions, biotic and abiotic elements and genetic systems (Wyatt, 1983; Charlesworth and Charlesworth, 1987; Arroyo and Squeo, 1990). Species' floral characteristics have been used to elucidate evolutionary relationships among angiosperms (Anderson and Hill, 2002; Rudall et al., 2002). The inflorescence and placentation types in *Solanum* species are of evolutionary significance. The model proposed by Rickett (1944) described the compound dichasium inflorescence type found in *S. erianthum* (a perennial tree) and *S. torvum* (a perennial shrub) as most primitive. Consequently, the simple dichasium type found in *S. aethiopicum* and *S. anguivi* can be regarded as diminutive compound dichasia, involving reduction of the axes. Reduction and projection of the axes in one direction produced a raceme, typical of *S. gilo* and *S. melongena.* Further reduction of axes and the attachment of pedicels at the same point produced an umbellate as evident in the two taxa of *S. scabrum*. Similarly, placentation has been used to assess relationships among angiosperms. The 2-carpel ovary is a primitive feature of *S. erianthum*. The subsequent growth of the septa gave rise to three, four or five carpels. The advanced 5 to 6 carpel ovary characterized the taxa of *S. scabrum* - a tetraploid. The placentation in Alliaceae was equally determined by the growth of septa. Absence of septa in *Gilliesia* and

Gethyum differentiates them from *Allium, Miersia* and *Solaria* (Ruddal et al., 2002).

The position and orientation of stamens in *Solanum* species allowed unimpeded access to pollen by variety of insect visitors. Similarly, the inflorescence of *Eriope blanchetii* did not present any mechanical obstacles to the insect pollinators (Da Silva et al., 2005). The flowers of *S. gilo* and *S. melongena* complex are large, brightly coloured and attractive to insect visitors. The flowers of the other species though small, are white and conspicuous. Unlike other species, the insect visitors did not discriminate between the flowers of *S. gilo* and *S. melongena*. Thus, the large flowers permitted repeated visits, elaborate interaction and intense probing by insect visitors, while flowers remained open. It had been noted that an enlarged and bulbous corolla allowed for greater number of pollinators, intra-plant interaction and gene flow (Thompson, 2000; Williams et al., 2001; Galloway et al., 2002; Mitchell et al., 2004). *Delphinum barbeyi* received more insect pollinators than its sympatric species, *Delphinum nuttallianum*, due to elaborate and bright coloured inflorescence (Williams et al., 2001). Floral architecture and display have been linked to the degree of mutualism existing between a species and its biotic community (Goulson, 2000; Da Silva et al., 2005). The pattern of insect visits to both *S. gilo* and *S. melongena* might have promoted greater interspecific interaction and frequent gene exchange, hence, their close morphology and preference for similar habitat. The low preference by insect visitors to the flowers of *S. anguivi, S. torvum*, *S. erianthum* and *S. scabrum* could be due to their small flower size which may limit the degree of insect interaction. However, insects repeatedly visited the flowers, seemingly unaffected by the magnitude of floral displays but guided rather by the need for pollen reward. Such, pollinator behaviour was also observed by Goulson (2000) and Ohashi and Yahara (2001). Repeated pollinator visits to a flower increase seed set and genetic diversity among progeny (Karron et al., 2006; Sahli and Coner, 2007).

The flowering periods were synchronised in all the species and they remained in flower till the end of the growing season. Pollen shed and stigma receptivity equally overlapped. These provided added opportunity for intra- and inter-specific crosses and assurance of high pollen load supply for effective pollination in the species Leading to natural occurrence of inter-specific hybrids (Amnuddin et al., 1985; Omidiji, 1983; Ughorgho and Oyelana, 1999). Some species of angiosperm, however, evolved different periods of flowering to avoid competition for pollinators with other closely related species. *Hamamelis virginiana* flowers in the fall to avoid competition with other species which flower from late winter to early summer (Anderson and Hill, 2002). The time and duration (10 h) of flower opening was extensive and greatly overlapped. This might suggest longer female phase for the genus and hence high pollination efficiency in these species. As an advantage, the unpollinated flowers received a second round of insect visitation when they reopened the next day. Thus, the length of female phase was mostly terminated at receipt of pollen in the species. Cruden et al. (1984) associated pollination efficiency to longevity hinged on the length of the female phase.

The short visit made by pollinators to the flowers of the different species might be indicative of the low level or minimal rewards offered. The same reason might be adduced for the seemingly lack of interest shown by insects to already visited flowers. Also, the single-source resource-offer by the different species, chiefly pollen, might also contribute to the reduction in number of visits and diversity of insect pollinators. The extent of visit and diversity of pollinators have been associated with the quality of rewards offered by individual plants (Matsumura and Washitani, 2000; Klinkhamer et al., 2001; Potts et al., 2004). Other limiting factor includes competition with other members of angiosperm species within the species' habitat range, as pollinators were generally unpredictable and non-specific. Non-specialized floral structure (*S. gilo* and *S. melongena*) was thought to attract diverse insect pollinators (Smith-Ramirez et al., 2005); however, only three insect species were active pollinators in these species. This may point to close genetic system and/or secretion of similar hormones and reward offer. Conversely, non-generalized, specialized pollination system exists where pollinators are predictable and abundant (Williams, 2004).

There was correlation between pollination efficiency and degree of pollen viability. *S. torvum* and *S. scabrum 'Erectum'* which had the highest pollen viability set more fruits than *S. gilo* with the lowest viability rate. *S. melongena* 'Golden' and *S. erianthum* were negatively correlated. The activities of the insect pollinators were complementary and effective at ensuring adequate fruit set in the latter species in spite of their low viability and pollen load. The complementary role of insect pollinators in achieving reproductive success and fruit production in angiosperm has been highlighted (Matsumura and Washitani, 2000; Pellegrino et al., 2005). The destructive activities of the larvae of *Anasa tristis* also contributed to the low fruit set in *S. gilo* and *S. melongena* complex. Floral and seed predation had also been observed in *Erythrina falcate* (Etchevery and Aleman, 2005).

Inbreeding characterized the *Solanum* species studied. The high failure rate of hybridization involving tetraploid *S. scabrum* confirmed it as an obligate inbreeder. *S. melongena* complex on the other hand, was highly fertile but with differing breeding and evolutionary mechanisms. While both were self compatible, *S. melongena* 'Melongena' was, in addition, strongly cross breeding than the recalcitrant *S. melongena* 'Golden' among the diploid species. The average successful hybridization of the other diploid species indicates that they are also facultatively cross breeding. Improvement in pollination

efficiency under natural conditions from pollinator-excluded to open pollination experiment accounted for the significant role of insect pollinator. Optimum pollination from assisted hand-selfed flowers further confirmed a potential pivotal role for insect pollinators. However, the low number of seed set in fruits from hybridized flowers showed an inherent reproductive barrier. Nevertheless, some successful experimental crosses had produced hybrids between related diploid species (Omidiji, 1983; Oyelana and Ogunwenmo, 2009; Ugborogho and Oyelana, 1999). Equally, natural hybrids are possible with the aid of insect pollinators. The existence of these natural hybrids including few triploids (Omidiji, 1983) and tetraploids (Amnuddin et al., 1985) confirmed a breakthrough in the reproductive barrier by some insect visitors. The reopening of flowers following pollination failure also suggests that inbreeding was not sufficient for fertilization of the numerous ovules, hence, the need for complementary role of insect pollinators. The low number of seeds in fruits of bagged flowers vis-à-vis high seed number in hand self-pollinated flowers further affirmed these insects as potential agents for effective pollination. The activities of the insect pollinators may significantly reduce or eliminate inbreeding depression among the self-compatible species to ensure population heterogeneity.

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REFERENCES

Amnuddin A, Khan HS, Beg MZ (1985). Interrelationship between Indian tetraploid *Solanum nigrum* and *S. nodiflorum* - mutants of *Solanum nigrum complex.* Acta Bot. Indica 13:84-89.

Amoako J, Yeboah-Gyan K (1991). Insect Pollination of three Solanaceous vegetable crops in Ghana with special reference to the role of African honey bee (*Apis mellifera* Adansonii) for fruit set. Acta Hortic. 288:255-259.

Anderson GJ, Hill JD (2002). Many to flower, few to fruit; the reproductive biology of *Hamamelis virginiana* (Hamamelidaceae). Am. J. Bot. 89:67-78.

Armbruster WS (1988). Multilevel comparative analysis of the morphology, function, and evolution of *Dalechampia* blossoms. Ecology 69:1746-1761.

Arroyo MTK, Squeo F (1990). Relationship between plant breeding systems and pollination. In S Kawano (ed.) Biological approaches and evolutionary trends in plants, Academic Press, London, pp. 205-227.

Barrett SCH (2003). Mating strategies in flowering plants: the outcrossing-selfing paradigm and beyond. Philosophical Trans. R. Soc. London, B, Biol. Sci. 358:991-1004.

Barrett SCH, Harder LD (1996). Ecology and evolution of plant mating. Trends Ecol. Evol. 11:73-79.

Barrett SCH, Harder LD, Worley AC (1996). The comparative biology of pollination and mating in flowering plants. Philosophical Trans. R. Soc. London 351:1271-1280.

Charlesworth D, Charlesworth B (1987). Inbreeding depression and its evolutionary consequences. Ann. Rev. Ecol. Syst. 18:237-268.

Cruden RW, Hermanut ZL, Shuttleworth J (1984). The pollination biology and breeding system of *Monarda fistulosa* (Labiatae). Oecologia 64:104-114.

Da Silva FO, Viana BF, Jacobi CM (2005). Floral biology of *Eriope blanchetii* (Lamiaceae) in coastal sand dunes of NE Brazil. Aust. Ecol. 30:243-249.

Devy MS, Davidar P (2003). Pollination systems of trees in Kakachi, a mid-elevation wet evergreen forest in Western Ghats, India. Am. J. Bot. 90:650-657.

Etchevery AV, Aleman CET (2005). Reproductive biology of *Erythrina falcate* (Fabaceae: Papilionoideae). Biotropica 37:54-63.

Fishbein M, Venable DL (1996). Diversity and temporal change in the effective pollinators of *Asclepias tuberosa*. Ecology 77:1061-1073.

Galen C, Cuba J (2001). Down the tube: pollinators, predators, and the evolution of flower shape in the alpine skypilot, *Polemonium viscosum*. Evolution 55:1963-1971.

Galloway LF, Crigliano T, Gremski K (2002). The contribution of display size and dichogamy to potential geitonogamy in *Campanula americana*. Int. J. Plant Sci. 163:133-139.

Goulson D (2000). Why do pollinators visit proportionally fewer flowers in large patches? Oikos 91:485-492.

Gruben GJH, Denton OA (2004). Plant resources of tropical Africa 2. Vegetables. PROTA Foundation, Wageningen, Netherlands/Backhuys Publishers, Leiden, Netherlands/CTA, Wageningen, Netherlands.

Harder LD, Barrett SCH (1996). Pollen dispersal and mating patterns in animal-pollinated plants. In Lloyd DG, Barrett SCH (eds.) Floral biology: studies on floral evolution in animal pollinated plants, Chapman and Hall, New York, pp. 140-190.

Herrera CM (1988). Variation in mutualisms: the spatiotemporal mosaic of a pollination assemblage. Biol. J. Linn. Soc. 35:95-125.

Ivey CT, Martinez P, Wyatt R (2003). Variation in pollinator effectiveness in swamp milkweed, *Asclepias incarnata* (Apocynaceae). Am. J. Bot. 90:214-225.

Johnston MO (1991). Pollen limitation of female reproduction in *Lobelia cardinalis* and *L. siphilitica*. Ecology 72:1500-1503.

Johnston MO (1998). Evolution of intermediate selfing rates in plants: pollination ecology versus deleterious mutations. Genetica 102-103: 267-278.

Karron JD, Mitchell RJ, Bell JM (2006). Multiple pollinator visits to *Mimulus ringens* (Phrymaceae) flowers increase mate number and seed set within fruits. Am. J. Bot. 93:1306-1312.

Klinkhamer PGL, De Jong TJ, Linnebank LA (2001). Small-scale spatial patterns determine ecological relationships; an experimental example using nectar production rates. Ecol. Lett. 4:559-567.

Kowalska G (2008). Flowering Biology of Eggplant and procedures intensifying fruit set. Rev. Acta Sci. Pollut. Hortorum Cultus 7(4):63-76.

Maloof J, Inouye DW (2000). Are nectar robbers cheaters or mutualists? Ecology 81:2651-2661.

Matsumura C, Washitani I (2000). Effects of population size and pollination limitation on seed-set of *Primula sieboldii* populations in a fragmented landscape. Ecol. Res. 15:307-322.

Mayfield MM, Waser NM, Price MV (2001). Exploring the ‘‘most effective pollination principle’’ with complex flowers: bumblebees and *Ipomopsis aggregata*. Ann. Bot. 88:591-596.

Mitchell RJ, Karron JD, Holmquist KG, Bell JM (2004). The influence of *Mimulus ringens* floral display size on pollinator visitation patterns. Funct. Ecol. 18:116-124.

Mitchell RJ, Karron JD, Holmquist KG, Bell JM (2005). Patterns of multiple paternity in fruits of *Mimulus ringens* (Phrymaceae). Am. J. Bot. 92:885-890.

Moeller DA (2005). Pollinator community structure and sources of spatial variation in plant-pollinator interactions in *Clarkia xantiana* ssp. *xantiana*. Oecologia 142:28-37.

Ohashi K, Yahara T (2001). Behavioural responses of pollinators to variation in floral display size and their influences on the evolution of floral traits. In Chittka L, Thomson LD (eds.), Cognitive Ecology of Pollination: Animal Behavior and Floral Evolution, Cambridge University Press, New York, pp. 274-296.

Omidiji MO (1983). Cytotaxonomic studies of *Solanum aethiopicum* x *S. macrocarpon* F_1 hybrid. Cytologia 48:35-40.
Oyelana OA, Ogunwenmo KO (2009). A derived pentaploid hybrid from *Solanum macrocarpon* L. (Solanaceae) and its induced multiploid mutant. J. Biol. Sci. 9(1):21-28.
Pellegrino G, Gargano D, Noce ME, Musacchio A (2005). Reproductive biology and pollinator limitation in a deceptive orchid, *Serapias vomeracea* (Orchidaceae). Plant Species Biol. 20:33-39.
Pettersson MW (1991). Pollination by a guild of fluctuating moth populations: option for unspecialization in *Silene vulgaris*. J. Ecol. 79:591-604.
Potts SG, Vulliamy B, Roberts S, Toole CO, Dafni A, Neemanand G, Willmer PG (2004). Nectar resource diversity organizes flower-visitor community structure. Entomol. Exp. Appl. 113:103-107.
Rickett W (1944). The classification of inflorescences. Bot. Rev. 10:187-231.
Rudall PJ, Bateman RM, Fay MF, Eastman A (2002). Floral anatomy and systematics of Alliaceae with particular reference to *Gilliesia,* a presumed insect mimic with strong zygomorphic flowers. Am. J. Bot. 89:1867-1883.
Sahli HF, Conner JK (2007). Visitation, effectiveness, and efficiency of 15 genera of visitors to wild Raddish, *Raphanus raphanistrum* (Brassicaceae). Am. J. Bot. 94:203-209.
Schemske DW, Horvitz CC (1989). Temporal variation in selection on a floral character. Evolution 43:461-465.
Smith-Ramirez C, Martinez P, Nunez M, Gonzalez C, Armesto JJ (2005). Diversity, flower visitation frequency and generalism of pollinators in temperate rain forest of Chiloe Island, Chile. Bot. J. Linn. Soc. 147:399-416.
Thompson JD (2000). How do visitation patterns vary among pollinators in relation to floral display and floral design in a generalist pollination system? Oecologia 126:386-394
Ugborogho RE, Oyelana OA (1993). Floral biology of six taxa of *Amaranthus* L. (Amaranthaceae) in Nigeria. J. Agric. Sci. Technol. 3:61-67.
Ugborogho RE, Oyelana OA (1999). A cytogenetic assessment of sterility in F1 hybrid and its backcross from *Solanum gilo* × *S. aethiopicum* (Solanaceae). J. Sci. Res. Dev. 4:61-70.
Vesprini JL, Galetto L (2000) The reproductive biology of *Jaborosa integrifolia* (Solanaceae): why are its fruits so rare? Plant Syst. Evol. 225:15-28.
Vogler DW, Kalisz S (2001). Sex among the flowers: the distribution of plant mating systems. Evolution 55:202-204.
Waites AR, Agren L (2006). Stigma receptivity and effects of prior self-pollination on seed set in tristylous *Lythrum salicaria* (Lythraceae). Am. J. Bot. 93:142-147.
Waser NM (1983). The adaptive nature of floral traits: ideas and evidence. In Real LA (ed.) Pollination biology, Academic Press, New York, pp. 241-285.
Wiggam S, Ferguson CJ (2005). Pollinator importance and temporal variation in a population of *Phlox divaricata* L. (Polemoniaceae). Am. Midl. Nat. 154:42-54
Williams CE (2004). Mating system and pollination biology of the spring-flowering shrub, *Dirca palustris*. Plant Species Biol. 19:101-106.
Williams CF, Ruvinsky J, Scott PE, Hew DK (2001). Pollination, breeding system and genetic structure in two sympatric Delphinium (Ranunculaceae) species. Am. J. Bot. 88:1623-1633.
Wilson P, Thomson JD (1991). Heterogeneity among floral visitors leads to discordance between removal and deposition of pollen. Ecology 72:1503-1507.
Wyatt R (1983). Pollinator- plant interactions and the evolution of breeding system. In Real LA (ed.) Pollination Biology, Academic Press, New York, pp. 51-95.