

Snail-pollination in *Volvulopsis nummularium*

Khoisnam Sarma¹, Rajesh Tandon^{1*},
K. R. Shivanna² and H. Y. Mohan Ram³

¹Department of Botany, University of Delhi, Delhi 110 007, India

²Ashoka Trust for Research in Ecology and the Environment,
659 'A' Main Road, Hebbal, Bangalore 560 024, India

³No. 194, SFS Flats, Mukherjee Nagar, Delhi 110 009, India

Pollination is a crucial ecological process that aids sexual reproduction in flowering plants. Although a variety of animals are known to bring about pollen transfer, pollination by snails (malacophily) has remained a rare and obscure phenomenon. Here we conclusively demonstrate the incidence of malacophily in *Volvulopsis nummularium* (family Convolvulaceae, commonly known as the morning glory family), a prostrate rainy-season weed, which is also visited by honey bees. Flowers open in the morning and last only for half a day. *Apis cerana indica* and Graceful Awlsnail (*Lamellaxis gracile*) are the pollinators. Snails are exclusive pollinators on rainy days, when bees are not active. Contrary to the belief that snails are destructive, we found that they do not affect the natural fecundity of *V. nummularium*. Manual-pollinations indicated that the plants were facultative autogamous. Pollination in *V. nummularium* by snails and honey bees represents an interesting guild, which is of adaptive significance in achieving high reproductive success without resorting to obligate selfing.

Keywords: Honey bees, malacophily, reproduction, snails, *Volvulopsis nummularium*.

POLLINATION is a significant process and one of the prerequisites for ensuring fruit- and seed-set in all sexually reproducing seed plants¹⁻³. A great majority of plants are pollinated by animals⁴ and only a few involve abiotic agencies^{1,5-7}. Among the zoophilous species, nearly 80% are pollinated by insects. The remaining involve a variety of birds, reptiles and mammals⁸⁻¹¹. Pollination by snails and slugs (malacophily) is a rare and infrequent phenomenon; so far it has been reported in seven species: *Rohdea japonica*, *Philodendron pinnatifidum*, *Colocasia odora*, *Calla palustris*, *Lemna minor*, *Chrysosplenium alternifolium* and *Phragmipedium caudatum*¹²⁻¹⁴. Prostrate habit of the plant and floral arrangement in which the stigma and anthers do not extend much beyond the corolla, are believed to be conducive to malacophily¹². However, some investigators doubt the possibility of snails or slugs being successful pollinators, and consider malacophily to be 'notorious and obscure'¹⁵ or even 'ridiculous'¹⁴.

Snails are usually active at night and also during the day in the rainy season. In an unexpected field observation, we found mass floral foraging by the terrestrial gar-

den snail – the Graceful Awlsnail (*Lamellaxis gracile*) on a common garden weed, *Volvulopsis nummularium*, in which flowers open in the morning and close by noon. This is a small (~15 mm in length) terrestrial snail commonly found throughout India and other tropical and sub-tropical parts of the world, except Australia¹⁶. We made a detailed investigation on the pollination ecology and breeding system of *V. nummularium* to ascertain whether (i) snails have a definite role in pollination, (ii) their exclusive foraging could induce fruit and seed-set and (iii) phytophagy by snails is destructive to the natural fecundity of this plant.

V. nummularium (L.) Roberty (syn. *Evolvulus nummularium* L., family Convolvulaceae), a native of tropical America, is a weed with prostrate and creeping habit that has naturalized in moist places in several parts of India¹⁷. The plants are multi-stemmed and propagate profusely through seeds as well as by vegetative means. Profuse growth of neighbouring individuals leads to the formation of expanded mats with overlapping stems. The plant becomes conspicuous when it starts blooming with the onset of monsoon (Figure 1 a). Peak flowering is reached by mid-August and lasts for a month. White, short tubular flowers are borne solitarily, and sometimes two in number, in the leaf axil. There is no previous report about any specific pollinator of *V. nummularium*.

A general survey of the distribution of plants of *V. nummularium* was carried out in Delhi region (28°12'–28°53'N, 76°50'–77°23'E) during May–June 2006. For the present investigation, ten sites in different undisturbed localities were randomly chosen for detailed observations. Phenoevents such as time of anthesis, anther dehiscence, flower longevity, period of flowering and fruiting were recorded. Anther dehiscence was determined by examining flowers ($n = 30$) under a stereomicroscope at different times, from the bud stage until anthesis between 0400 and 0800 h. Commencement of stigma receptivity and its duration were recorded through semi-vivo pollination studies¹⁸. Ovule production in a pistil was computed by scoring the cleared pistils ($n = 30$). Average pollen production in a flower ($n = 30$) was estimated using a haemocytometer¹⁹. Fertility of pollen grains was tested by scoring their stainability with 1% acetocarmine¹⁸. Pollen viability was assessed through fluorochromatic reaction test²⁰. Pollen viability period was determined using pollen grains sampled every 60 min following anther dehiscence, till it declined to zero. The nature of reserve material in the pollen grains was identified using Sudan black for lipids and potassium iodide for starch.

As the flowers were visited only by snails and honey bees, detailed field observations were confined to these foragers. The standard entomological method was employed for collecting and preserving the honey bees²¹. To prevent the drying of snails during sampling, they were narcotized by asphyxiation, followed by washing thoroughly with distilled water to remove the mucous, passed

*For correspondence. (e-mail: rjtnd@rediffmail.com)

through ascending ethanol series (20, 40, 60 and 70%) and finally stored in 70% ethanol¹⁶ for identification. The samples were sent to the Zoological Survey of India, Kolkata for identification.

The role of floral visitors in pollination and their efficiency was ascertained on the bases of foraging behaviour, flower-handling time, pollen load and the number of pollen grains deposited on the stigma after their visit. For computing the stigmatic pollen load on the freshly opened flowers and after the visits of the foragers, flowers ($n = 20$, each site) were collected in dry screw-cap vials (2 ml), and their stigmas were mounted in a drop of aur-

amine O' and observed under an epifluorescence microscope (Nikon, AXII Optiphot). Foraging period and flower-handling time were recorded separately for each forager from all the study sites (a total of 90 h of observation). The average amount of pollen load on each floral forager (snails, $n = 300$; honey bees, $n = 200$) was counted by removing the pollen grains with a brush on separate clean microslides under the stereomicroscope (Nikon, SMZ 800), staining with auramine O' and examining them under the epifluorescence microscope.

To ascertain whether or not the visit of snails and bees could lead to subsequent fruit- and seed-set, the flowers

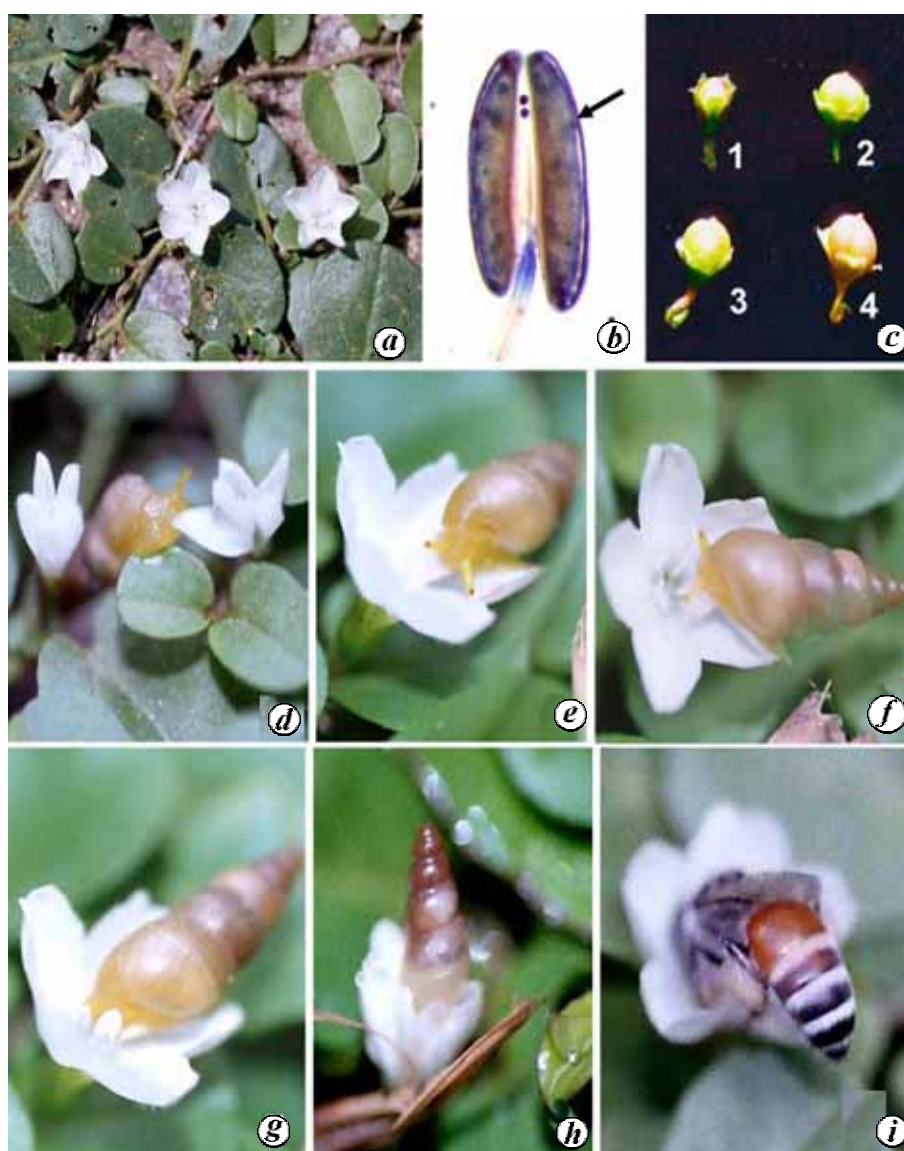


Figure 1. *a*, Close-up of *Volvulus nummularium* plant showing prostrate habit with open flowers. *b*, Whole mount of a stamen showing latrorse (arrow) longitudinal line of dehiscence of the anther. *c*, Fruits with variable number of seeds (1–4). Fruit size is proportional to seed number. *d–g*, Pollination mechanism in *V. nummularium*. *d*, A snail approaches the flowers at anthesis. *e*, It gradually crawls towards the anthers to forage. *f*, The shell comes in contact with the dehiscent anthers and pollen grains get adhered to the last whorl. *g*, Pollen transfer occurs when the snail visits the next flower. *h*, A snail entering a partially opened flower during a rainy day. Note vertical orientation of the shell. *i*, A honey bee foraging the flower.

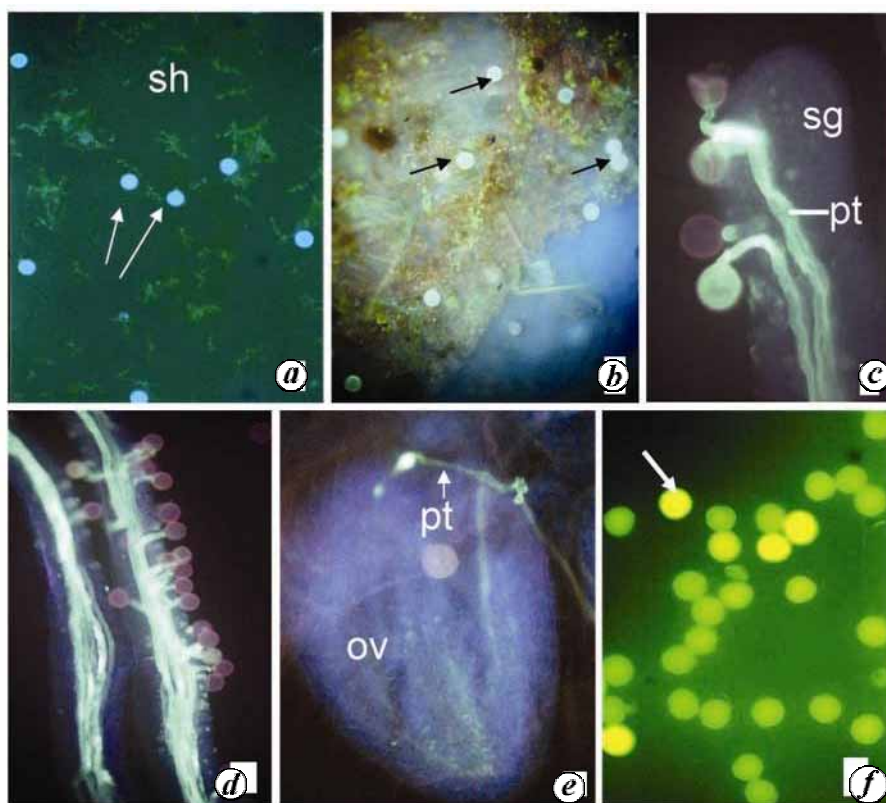


Figure 2. *a–f*, Epifluorescence photomicrographs. *a*, Auto-fluorescing pollen grains on the shell (sh) of a snail. *b*, Freshly ingested pollen (arrows) in the gut of the snail. *c*, *d*, Pollen germination on the stigma (sg), and pollen tube (pt) growth after pollination by snail and honey bee respectively. *e*, Entry of pollen tube (pt) into the ovule (ov) after hand-self pollination. *f*, Pollen viability after 5 h; only the brightly fluorescing pollen grains (arrow) are viable.

were bagged after their first visit ($n = \sim 500$ each) and kept under observation for fruit-set. Interfloral movement of the foragers in a flowering patch was independently analysed for snails and bees on separate occasions (at five study sites) over a period of two months. Honey bee activity was traced by following the individual bee ($n = 200$) till it left a patch of plants, whereas the snails were marked with a dot of red on their shells for following their interfloral movement. The extent of phytophagy by snails was ascertained by recording the type of floral parts consumed, and also by dissecting the snails and examining the contents of their gut under the microscope.

Fruit- and seed-set through open pollination was computed by tagging the floral buds ($n = 540$) 12 h before anthesis. Any incidence of apomixis was ascertained by bagging (with transparent paper bags, 4×2 cm) the emasculated flowers ($n = 220$) 12 h before anther dehiscence. For assessing spontaneous autogamy, flowers were bagged without emasculation. Hand-self (forced autogamy) and cross-pollinations (xenogamy) were performed to ascertain the extent of self-incompatibility, if any, in the species. Self-compatibility index (SCI)²² was computed based on these results. For all statistical purposes, SPSS12 was used. One-way ANOVA was employed for determin-

ing any significant difference in fruit- and seed-set between pollination treatments. The percentage values were arc-sine transformed before subjecting to ANOVA.

Flowering in *V. nummularium* occurred in a synchronous and seasonal manner at all the study sites. On bright and sunny days, flowers anthesized between 0700 and 0800 h, and remained open until noon. On rainy days, anthesis was delayed by ~ 60 min. and the flowers remained only partly opened (Figure 1 *h*). The corolla was slightly twisted in the bud and after complete opening, it assumed a sub-rotate shape (Figure 1 *a*, *e–g*). The corolla measured 6.66 ± 0.28 mm across in a fully opened flower. Flowers were odourless and lacked nectar.

During anthesis, five epipetalous stamens diverged from the throat of the corolla tube. The pistil is made up of a superior, unilocular ovary with four ovules borne on the basal placenta, and an inconspicuous style with a four-lobed stigma (6.2 ± 1.3 mm in length). Anther dehiscence occurs simultaneously with anthesis by a longitudinal line of dehiscence. The pollen grains were presented latrosely (Figure 1 *b*). On an average, each flower bore 968 ± 12 pollen grains and $\sim 96.3\%$ of them were fertile. The fresh pollen grains were primarily lipidic, sticky and exhibited 93% viability. Viability dropped to $<3\%$ within 5 h (Figure 2 *f*).

Table 1. Comparison of results of fruit- and seed-set following different modes of pollination

Pollination treatment	% Fruit-set (<i>n</i>)	<i>F</i>	Average number of seeds in a capsule (<i>n</i>)	<i>F</i>
Open-pollination	74.5 (540)	—	2.60 ± 0.04 (402)	—
Spontaneous autogamy	51.8* (158)	4.67	1.73 ± 0.11* (82)	2.03
Manual self-pollination (forced autogamy)	70.6* (551)	3.28	2.32 ± 0.04* (389)	1.98
Manual cross-pollination (xenogamy)	82.7* (503)	1.18	3.3 ± 0.06* (416)	0.06
Snail-pollination (malacophily)	74.4 (504)	n.s.	3.4 ± 0.06* (375)	1.26
Bee-pollination (melittophily)	89.4* (503)	0.88	3.6 ± 0.08* (450)	1.01

*Values significantly different at $P = 0.05$, when independently compared with open pollination using one-way ANOVA; $df = 9$; n.s., Difference with open-pollination not significant.

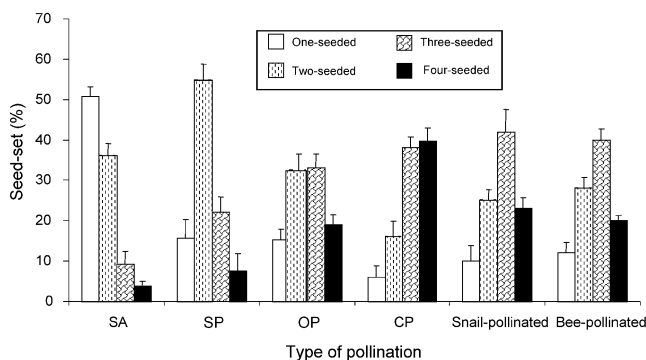


Figure 3. Effect of type of pollination on fruits bearing 1, 2, 3 or 4 seeds (percentage). (SP, Manual self-pollination; SA, Spontaneous autogamy; OP, Open-pollination; CP, Manual cross-pollination).

The stigma lobes were dry and non-papillate²³. Stigma receptivity was attained before the opening of flowers at 0630 h and lasted till 1130 h. The pollen to ovule ratio was 241 : 1.

Flowers were visited by the terrestrial garden snail (*L. gracile*; Figure 1 d–h), and honey bees (*Apis cerana indica*; Figure 1 i). The population size of snails in the soil in which the plants were growing was approx. 30–50 per sq. m. Foraging activity of both animals resulted in pollen transfer to the stigma, pollen germination and eventual fertilization and fruit-set (Figures 2 c–e and 3).

On sunny days foraging occurred in a successive manner; snails came first followed by honey bees. Snails foraged flowers between 0700 and 0900 h, and each snail spent 10 ± 3.3 min in a flower ($n = 43$). While consuming the pollen grains and anthers, the head, foot and broader portion of the shell (last whorl) of the snail came in contact with the dehiscent anthers and stigma lobes, and effected pollen transfer (Figure 1 e, f). Foraging behaviour, gut analysis after fresh foraging of flowers (Figure 2 b) and faecal analysis showed that the snails primarily consumed

the whole stamen or only the pollen grains. A snail carried approx. 180 ± 14.2 ($n = 87$) pollen grains on its shell (Figure 2 a). The average pollen count on the stigma of the snail-visited flowers ($n = 30$) was 95 ± 8.7 . 75% ($n = 504$). Nearly 74% of snail-visited flowers developed into fruits (Table 1).

Honey bees visited the flowers between 0800 and 1100 h. On an average a honeybee spent only a brief period (4.2 ± 1.26 s) in a flower. Interestingly, the bees completely avoided flowers being foraged by snails or those that had been previously visited by the snails. However, the reverse was not true; snails readily foraged bee-visited flowers. Honey bees collected the pollen grains sternotribically on their thorax and hind legs in the form of pollen baskets. On an average each bee carried 2516.8 ± 47.64 pollen grains on its body parts. The stigmas of honey bee-visited flowers ($n = 45$) showed 123 ± 17 pollen grains and ~90% fruit-set occurred in the honey bee-visited flowers ($n = 503$ flowers; Table 1).

On rainy days, anthesis was delayed by 30 min and flower buds opened only partially. On such days, honey bee activity was totally absent. However, snails were active and forcibly entered the partly opened flowers (Figure 1 h) and foraged for a longer duration (18 ± 5.6 min, $n = 28$ flowers) than usual.

Flowers that were bagged after emasculation showed no fruit-set, thus ruling out pseudogamous form of apomixis. However, fruit-set resulted from other modes of controlled pollination (Table 1). The average number of pollen grains on the stigma of flowers that were bagged to assess spontaneous autogamy was as low as 11.2 ± 1.7 ($n = 37$) and spontaneous autogamy resulted in nearly 51% fruits ($n = 158$ flowers), with a seed-set of 1.73 ± 0.11 per capsule (Table 1). The percentage fruit-set through spontaneous autogamy was significantly lower than that resulting from other modes of pollination. Similarly, manual autogamy resulted in considerably lower seed-set in a

capsule than that resulting from open-pollination and xenogamy. The SCI value was 0.7.

The number of seeds ranged from one to four in each capsule (Figure 1 c). Based on the proportion of seeds in a fruit, the response among different pollination categories that yielded fruits was variable (Figure 3). The proportion of three- and four-seeded fruits was highest in manually cross-pollinated flowers (Figure 3). The difference in seed-set pattern between malacophily and melittophily was statistically insignificant.

We have clearly established the incidence of snail pollination in *V. nummularium* based on: (i) presence of a large number of snails in soil inhabited by the plants, (ii) foraging pattern of the snails, (iii) presence of pollen load on their body parts, (iv) occurrence of a larger number of pollen grains on the stigma of snail-visited flowers compared to natural autogamy, and (v) development of a high proportion of fruits and seeds in snail-visited flowers. These studies adequately fulfil the requirements of the postulates^{24,25} formulated to establish whether or not a flower-visitor is a pollinator. The habit and habitat of the plant and the presentation of essential organs in the flowers conform to the floral traits suggested for malacophilous pollination syndrome^{12,13,15}. Analysis of freshly digested floral parts in the gut of the snails and their faecal analyses clearly demonstrated their preference for pollen grains/ anthers. Importantly, as the stigmatic lobes or other parts of the pistil are not consumed by snails, opportunity for pollination and fertilization is not diminished. Thus, snail-pollination is not destructive to the natural fecundity of *V. nummularium*.

Lipidic pollen was the sole floral reward for honey bees, whereas snails foraged pollen as well as anthers. Avoidance of snail-visited flowers by bees could be due to the lack of a landing platform or absence of floral reward. It is also likely that the slimy trail left by a snail on the flower could have dissuaded the bees.

Values of pollen: ovule ratio²⁶ and SCI²² obtained through controlled pollinations, suggest that the mating system in the species is facultative autogamous. However, selfing is not sufficiently effective through the spontaneous mode. This is indicated by the poor pollen load on the stigmas of flowers not visited by any floral foragers and lower fruit and seed-set. These findings indicate that stigma lobes do not effectively come in contact with the laterosely dehiscent anthers. Pollination efficiency (stigmatic pollen load) is significantly increased with the participation of snails and honey bees. Although the laterose pollen presentation may serve as an important contrivance to prevent autogamy in hermaphroditic and self-compatible plants, in those species in which the pollen grains and the stigma are presented simultaneously, flower-visitors may cause 'facilitated selfing' and outcrossing or geitonogamy in a single pollination act²³. Such mixed deposition of pollen is likely to produce mixed progeny in the population. As the snails forage restrictively to patches below which they

inhabit, their pollination activity may result largely in either selfed or geitonogamous progenies. However, xenogamy may also be brought about by snails when branches from the neighbouring plants overlap, bringing together flowers of different plants. Xenogamy is expected to be more prevalent in honey bee-pollinated flowers.

Pollination in the family Convolvulaceae is primarily by bees, although there are instances of pollination by moths, birds and bats²⁷. The discovery of malacophily in *V. nummularium* is thus a novel addition to the pollination syndromes prevailing in the family. Pollination mechanism in *V. nummularium* represents an unusual guild that involves a polylectic interaction. Although snails are not the exclusive pollinators for ensuring fruit-set in the plants, they play a significant role in the pollination effort, especially on rainy days when the activity of bees is completely lacking. Adoption of snail as a pollinator, therefore, enables pollen transfer even on rainy days. We infer that these pollination strategies of natural autogamy to a limited extent followed by snail and/or bee pollination in *V. nummularium*, are of adaptive significance in achieving reproductive success without resorting to obligate selfing. This rare instance of malacophily points to the need to examine rainy-season flowering plants or those inhabiting water bodies more thoroughly, for a better understanding of the role of snails in pollination.

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ACKNOWLEDGEMENTS. We thank Dr Ramakrishna, Joint-Director, Zoological Survey of India, for identification of the snail and Ms Sanyukta Jaiswal for help. K.R.S. and H.Y.M. acknowledge the award of Senior Scientist and Honorary Scientist positions respectively, by the Indian National Science Academy, New Delhi.

Received 31 July 2007; revised accepted 3 August 2007