Pollinator-induced anther dehiscence in *Incarvillea emodi* (Bignoniaceae)

Male function in angiosperms refers to the production of viable pollen, its efficient dispersal to carry out effective pollination and successful completion of pollen–pistil interaction. The reproductive success of flowering plants depends on the availability of viable pollen for fertilizing their ovules and availability of resources for their successful maturation into seeds. In some angiosperms, anthers do not dehisce even though they carry viable pollen grains. This aberration, called functional male sterility[1], limits their reproductive success. Plants of *Incarvillea emodi* are functionally male sterile, since pollen discharge is not observed in bagged flowers at any stage prior to or after anthesis. Structural features of the anther – a spiny appendage present towards the base of the anther and filiform connective that keeps the two anthers, one on either side of the style, locked – impose this constraint. Although the flowers are chasmogamous, there is no evidence of pollen shedding even after anthesis. Pollen is, however, shed by the mediation of a specific pollinator, which pushes the spiny appendage at a specific stage of floral development. The plants depend upon the pollinator not only for pollen transfer but also for pollen discharge from anthers. Otherwise, the pollen grains remain trapped inside

the pollen sacs. This correspondence describes and discusses the insect-induced male function and its implications upon the reproductive success of the species.

Incarvillea emodi (Lindl.) Chaterji is reported from India, Pakistan, Afghanistan and western Nepal and listed as rare in India[2–4]. The plants grow along the rock crevices (Figure 1 *a*) and a single population of 35 plants scattered over 1 km^2 was located over a rocky terrain with steep mountain slopes in Nandani, 25 km north of Jammu city. The plants bear large pink, companulate flowers in long terminal racemes (Figure 1 *b*). An individual flower measures 4.88 ± 0.28 cm in length. The male reproductive apparatus consists of four epipetalous stamens, held firmly along the style in two opposite pairs by two pairs of filiform projections of the connective (Figure 2 *a–c*). The stamens are dimorphic, measuring 3.2 ± 0.11 and 2.6 ± 0.11 cm in length respectively. The anther lobes are covered at their back by a fleshy, turgid, light-green sheath that turns brown as the anther ages and shrivels. A prominent suture wherefrom pollen grains disperse, extends from the tip of anther and covers three-fourth length of the anther lobe. A prominent stiff, spiny appendage is present near the base of each anther in line

with the suture (Figure 2 *c*). Until anthesis, the spiny appendage remains bent at acute angle towards the tip of the anther (Figure 3 *a*). Within 24 h of anthesis, the spiny appendage becomes erect at right angle to the suture (Figure 3 *b*). The appendage turns towards the anther base 48 h post-anthesis and remains in this position for the rest of its life (Figure 3 *c*).

In order to monitor the movement of spiny appendage and study its impact on anther dehiscence, 50 flowers (ten each on five plants raised in the Botanical Garden, University of Jammu) were selected at random and covered with butter-paper bags 12 h prior to anthesis to exclude insects. These were divided into two sets of 25 flowers each, which were designated as A and B. From set A five flowers were excised 12 h after bagging when anthesis had just started, and ten flowers each were excised 36 and 60 h after bagging respectively. Stamens of the excised flowers were mounted on a slide and examined under a stereomicroscope for the posture of spiny appendage. Manipulation of 90 anthers, 30 each with spine in forward, erect and backward position, by applying pressure on the appendage, further revealed the relationship between appendage posture and anther dehiscence. Results of these manipulations are summarized below.

Figure 1 *a*, *b*. *In situ* growing plant of *Incarvillea emodi* (*a*) bearing flowers in dense racemes (*b*).
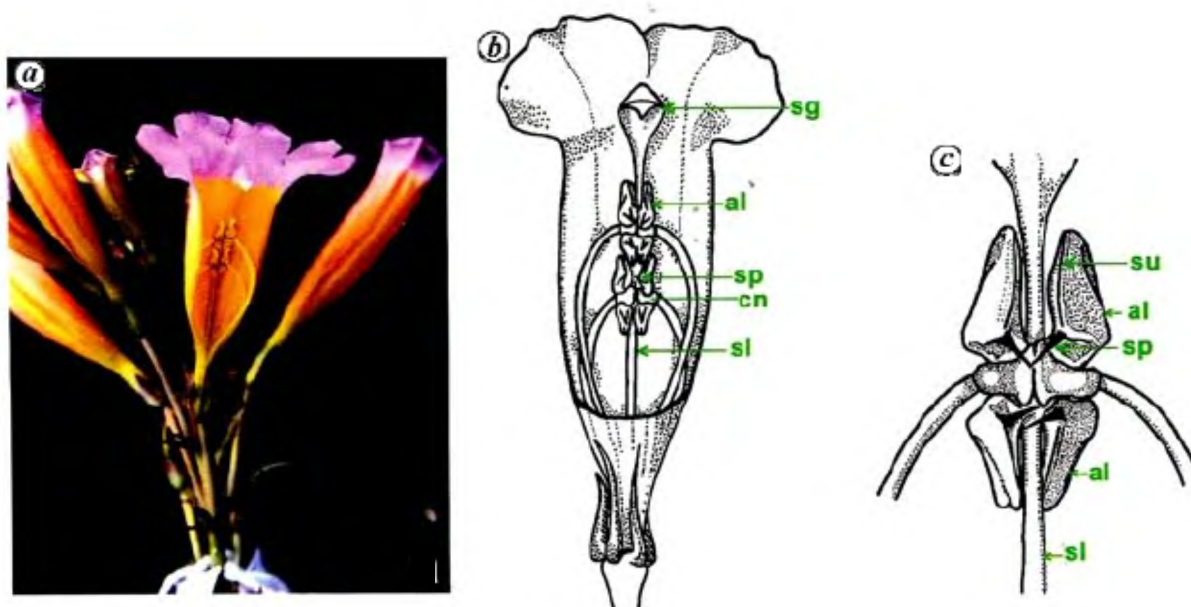
Figure 2 a–c. A slit open flower of *I. emodi* (*a*) showing the position of stamens vis-à-vis stigma and diagrammatic representation thereof (*b*). Note the anther lobes (al) tightly positioned along the style (sl) with the connective (cn). Spiny appendage (sp) present at the end of suture (su) towards the base of anther lobe (*c*).
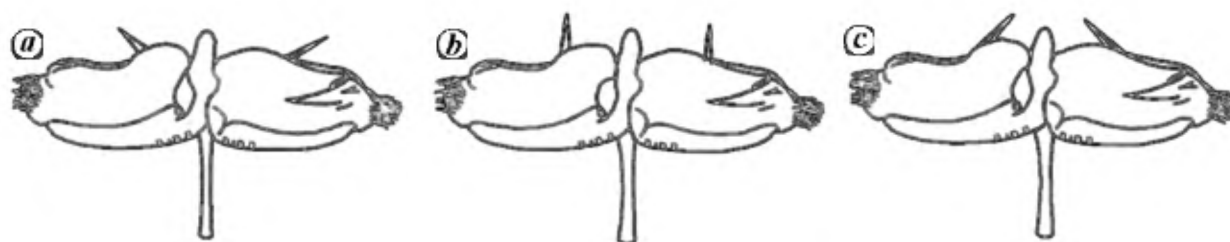

Figure 3 a–c. Anthers with the spine in different angles. Line drawings showing spine bent towards tip (*a*), at right angle (*b*) and towards the connective (*c*) of the anther.

At anthesis the spine is bent towards the tip of the anther and no amount of pressure applied induced anther dehiscence by opening the slit; after 24 h of anthesis, the spine assumed a right angle to the anther surface and when subjected to a little pressure led to the opening of the dehiscence slit and explosive release of pollen grains; after 48 h of anthesis it bent towards the connective and no amount of manual pressure caused opening of the dehiscence slit, leading to no pollen release.

Bags from flowers of set B were also removed at different intervals, i.e. five after 12 h and ten each after 36 and 60 h of bagging respectively. They were closely monitored for insect visitation. The interaction between insects and the spiny appendage, and the condition of dehiscence slit were examined immediately after insect visitation.

The stigmas excised from both *in situ* and *ex situ* habitats of the plants were scanned for pollen load and germination. Manual pollinations on plants raised in the University Botanic Garden at Jammu were carried out to assess the significance of insect-induced male function in the species. The fruit set was also compared between flowers subjected to open pollination both *in situ* and *ex situ* conditions.

In set A, anthers of flowers excised 12 h prior to anthesis invariably showed the spiny appendage bent forward, making an acute angle vis-à-vis the anther tip. Flowers observed 24 h after anthesis showed the position of the spine at right angle to the dehiscence slit. The flowers studied 48 h after anthesis showed the spine bent backwards towards the connective.

In set B, no insect visits the flower before and even 12 h after anthesis because the corolla is not fully expanded. Twenty-four hours after anthesis, three species of *Apis*, namely *A. florea*, *A. dorsata* and *A. mellifera* were seen hovering over the flowers. Only *A. mellifera* was seen entering the corolla tube. The same bee was observed visiting flowers after 48 h of anthesis. Insect visits are more frequent in the afternoon. The visits last till 17:30 h. After landing on the mouth of the corolla tube, the bee enters inside to forage the nectar concealed at its base.

Table 1. Results of pollination treatments in *Incarvillea emodi*

	Pollinator exclusion	Manual pollination using		Control (open pollination)	
		Self-pollen	Cross-pollen	*In situ*	*Ex situ*
Total number of flowers	133	221	120	748	847
Number of fruits set	Nil	221	120	191	379
Percentage fruit set	Nil	100	100	25.53	44.7
Percentage seed set	Nil	76.29	81.95	17.11	31.95

Individual visits to a flower are brief, lasting 40–50 s, very rarely extending to 2.5 min. Bees exiting from flowers 24 h after anthesis were trapped, etherized and studied under the stereomicroscope for pollen load on their body. They contained heavy load of conspecific pollen on different body parts (head, 33; thorax, 77; abdomen, 228; wings, 144; fore legs, 77; middle pair, 212; and hind legs, 214). Anthers excised from flowers after bee visitation were nearly empty and had their spine standing erect. Bees trapped while leaving the flowers, 48 h after anthesis had little pollen attached to their body, but such anthers were full of pollen.

Flowers of *I. emodi* are herkogamous. The bifid stigma is positioned above the anthers (Figure 1 *a*). As the insect moves towards the base of the corolla tube in search of nectar, it disturbs the upright, spiny appendage and in return receives considerable quantity of pollen deposited on its body, most of it on the abdomen. When such a pollen-loaded bee visits another flower of the same or different plant, the abdomen brushes against the bifid papillate stigma that it encounters first while entering the corolla tube, and brings about pollination.

The 133 flowers subjected to pollination exclusion by bagging them yielded no fruit, but all flowers subjected to manual self- and cross-pollination respectively, bore 100% fruit set (Table 1). There was significant difference in fruit set between open-pollinated flowers of plants growing *in situ* and *ex situ* (Table 1).

The mechanical process of pollen shedding in *I. emodi* is the result of the pressure exerted by the spiny appendage of the anther. Arrangement of anthers into stable pairs that are held appressed to the inner surface of the corolla tube contributes to the efficiency of pollen shedding. Such structural organization of the male reproductive apparatus facilitates insect-mediated pollen release. The visiting *A. mellifera* presses the erect spine while on its way down the corolla tube. The diameter of the insect body ensures application of pressure on the appendage, resulting in the opening of the pollen sacs and discharge of pollen. The size and structure of the insect body and that of *I. emodi* flowers provide for specific flower–insect interaction. The insect-mediated opening seems to be an adaptation to minimize pollen loss and by doing so assures efficient use of resources. Pollen discharge and subsequent pollination in *I. emodi* is obviously subservient to insect visitation. In the absence of any visitor, plants of *I. emodi* are as good as male sterile since their pollen remains locked inside the pollen sacs. Our observations on the visitation, behaviour and role of pollinator (*A. mellifera*) are based only on *ex situ*-raised plants.

Poor fruit set and, little or no pollen load on stigmas of plants growing in nature indicate pollinator limitation. Results of manual self- and cross-pollination further support the conclusion that flowers of *I. emodi* are obligatory entomophilous. The main reason behind low pollinator visitation in nature seems to be the reduced population size of the pollinator. The widely scattered plants (35 plants in 1 km^2 radius) provide little visual attraction to pollinators. Similar observations have been recorded by various workers[5–8].

1. Kaul, M. L. H., In *Male Sterility in Higher Plants*, Springer-Verlag, New York, 1988.
2. Sharma, B. M. and Kachroo, P., In *Flora of Jammu and Plants of Neighbourhood*, Bishen Singh Mahendra Pal Singh, Dehradun, 1981.
3. Goel, A. K. and Bhattacharaya, U. C., In *An Assessment of Threatened Plants of India* (eds Jain, S. K. and Rao, R. R.), Botanical Survey of India, Howrah, 1983, pp. 14–17.
4. Peterson, A., *Plantsman*, 1992, **13**, 199–202.
5. Hendrix, S. D. and Kyhl, J. F., *Conserv. Biol.*, 2000, **14**, 304–313.
6. Powell, A. H. and Powell, G. V. N., *Biotropica*, 1987, **19**, 176–179.
7. Jennersten, O., *Conserv. Biol.*, 1988, **2**, 359–366.
8. Van Swaay, C. A. M., *Biol. Conserv.*, 1990, **52**, 287–302.

SUSHEEL VERMA[1,*]
VEENU KAUL[2]
RANI MAGOTRA[2]
A. K. KOUL[2]

[1]Centre for Biodiversity Studies,
School of Biosciences and Biotechnology,
BGSB University,
Rajouri 185 131, India
[2]Department of Botany,
University of Jammu,
Jammu 180 006, India
*For correspondence.
e-mail: eremurus@rediffmail.com