Pak.J.Bot., 24(1): 79-87, 1992.

THE POLLINATION BIOLOGY OF *ARISTOLOCHIA BRACTEOLATA* LAMK. (ARISTOLOCHIACEAE).

MOMIN A. RAZZAK, TAHIR ALI AND S.I.ALI

Department of Botany,
University of Karachi, Karachi-75270. Pakistan.

Abstract

Aristolochia bracteolata Lamk. is partially protogynous. The flower exibits fly-trapping mechanism. Only one pollinator, belonging to the genus *Forcipomya* (Diptera; Ceratopogonidae) is involved. The pollen germination takes place within the dehisced anthers and on perianth parts i.e., within the trap and the prison, producing the pollen tubes, which ultimately reach the stigma and bring about fertilization. Breeding system study revealed that *Aristolochia* is self-compatible. Significant difference between treatments (bagged and open - pollinated flowers) in terms of fruit set, fruit and seed weight was observed. In all the 3 populations studied, difference in seed set and seed weight was significant while non- significant in fruit set and fruit weight.

Introduction

The genus *Aristolochia* L., is represented by c. 350 species distributed in tropics and subtropics (Willis, 1973). The available information indicates that the members of the genus share a peculiar mode of pollination. Flower perianth is specially modified to trap the insects. Protogyny is reported in all the cases so far studied. Insects, mostly Diptera, many of them covered with pollen from previous entrapment, enter the trap of the flower, which often has trichomes (permitting only one way traffic), and become trapped, where they pollinate the flower. The insects remain trapped untill the stamens ripen and cover (or load) the insects with pollen, the hairs in the trap wilt, allowing the insects to escape (Cammerloher, 1923, 1933; Graham, 1839; Lindner, 1928; Percival, 1965; Meeuse, 1961; Proctor & Yeo, 1973; Stebbins, 1974). Time of imprisonment of insects varies from species to species. Insects are attracted to *Aristolochia* flowers by their odour (Percival, 1965; Proctor & Yeo, 1973; Daumann, 1971; Brantjes, 1980). Flowers usually smell unpleasantly like sweat, faeces, urine and decaying protein (Percival, 1965; Proc tor & Yeo, 1973). Visitors reported for *Aristolochia* species consist mostly of Diptera of the families Bibionidae, Ceratopogonidae, Chiroonomidae, Chloropidae, Lauxanidae, Milichiidae, Mycetophilidae, Phoridae and Sepsidae (Cammerloher, 1933; Percival, 1965; Daumann, 1971; Brantjes, 1980). Breeding behaviour of *Aristolochia* is not fully explored and most of the work is confined to the structural complexity of flower perianth, regarding its fly trapping mechanism.

In Pakistan, only three species of *Aristolochia* are present (Qaiser, 1977). Of these, *A. bracteolata* and *A.punjabensis* are native whereas *A.elegans* is an exotic Brazilian species. As no work has been done on the floral biology of the local species, the account of the pollination biology of *A. bracteolata* is presented.

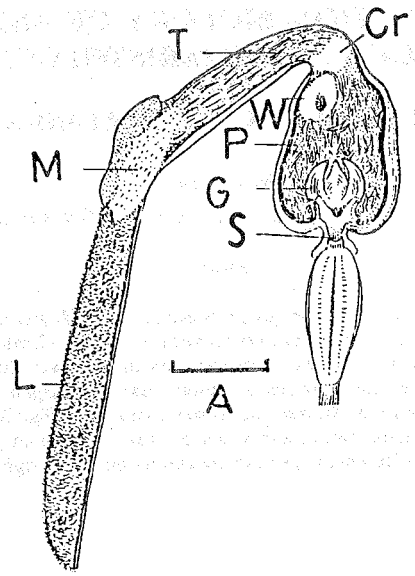

Fig.1. Flower with half of the perianth cut away. L: Lip. M: Mouth. T: Trap. Cr: Clear ring (devoid of hairs). W: Window. P: Prison. G: Gynostegium. S: Style (scale line = 4 mm).

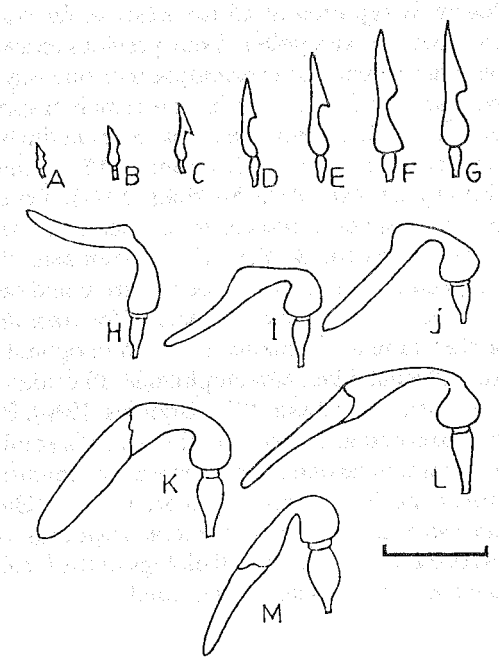

Fig.2. Daily observations on the developing bud. A-L. Different developmental stages from bud to mature flower. M. Old flower (scale line = 20 mm).

Materials and Methods

Populations growing in the following localities in Karachi, provided the material for these studies. (1) Karachi University Campus (KUC) (2) Pakistan Council of Scientific & Industrial Research Laboratories (PCSIR) (3) Near the National Institute of Public Administration (NIPA) and (4) Jinnah Post Graduate Medical Centre (JPMC).

Floral morphology and phenology: For the study of floral morphology and phenology, prior to anthesis, full-sized flower buds were marked. Ten flowers were taken from this 'pool', one every 4 h. over a 36 h. period. These were used to determine the different reproductive stages of the flower.

Type, Structure and Orientation of Hairs: Hairs were observed microscopically for their type, structure and orientation on the different parts of the perianth and stigmatic lobes.

Detection of Wax: The presence of wax was tested by Ether test (Johansen, 1940). Sections of the perianth were placed on glass slide and covered with the cover slip. The ether was introduced from one side of the cover slip. Later the ether was allowed to evaporate and the slide was observed microscopically.

Number of Pollen Grains/Flower: Undehisced anther was removed from the flower, placed on a glass slide and covered with the cover slip, then squashed in acetocarmine and the number of pollen grains/anther were counted microscopically. The total number of pollen grains/flower were calculated by multiplying the number of pollen grains/anther with the number of anthers/flower (i.e.6).

Odour: Osmophores (fragrance emitting glands) were detected by the method used by Vogel (1962). The flowers were dipped in the neutral red solution for about 30-40 minutes, washed in water and then observed under the microscope. Appearance of the reddish spots on different floral parts indicate the presence of osmophores.

Pollination Efficiency: Sixtyeight flowers were collected and placed in the Petri dishes (one flower per Petri dish) containing a small cotton ball moistened with acetic ninhydrin (the ninhydrin vapor was sufficient to kill the insects). After five minutes the (a) number of insects per flower was directly scored. (b) the pollen load per insect was determined and (c) the number of pollen grains on the stigmatic lobes was also determined microscopically in young and old flowers i.e., before and after anther dehiscence, respectively.

Breeding System: In order to determine the breeding system, mature buds from different plants in each of the three populations (PCSIR,NIPA and JPMC) were bagged (N = 50). At the same time other mature buds (N = 50) from different plants in each population were tagged and treated as open-pollinated flowers. After one month, fruits from bagged (selfed) and unbagged (tagged i.e. open pollinated) flowers were collected and Two Way - Analysis of variance was applied on fruit and seed set, fruit and seed weight between both the treatments and all the three populations.

Observation and Results

Floral morphology and phenology: Following parts of the perianth may be recognised (a) lip (b) mouth (c) trap and (d) prison. There are six sessile stamens forming part of the gynostegium; the ovary is inferior with a short style and 6-lobed stigma (Fig.1).

Formation of the flower from the youngest bud takes 11-12 days (Fig.2). After anthesis, the following four stages of the flower may be recognised:

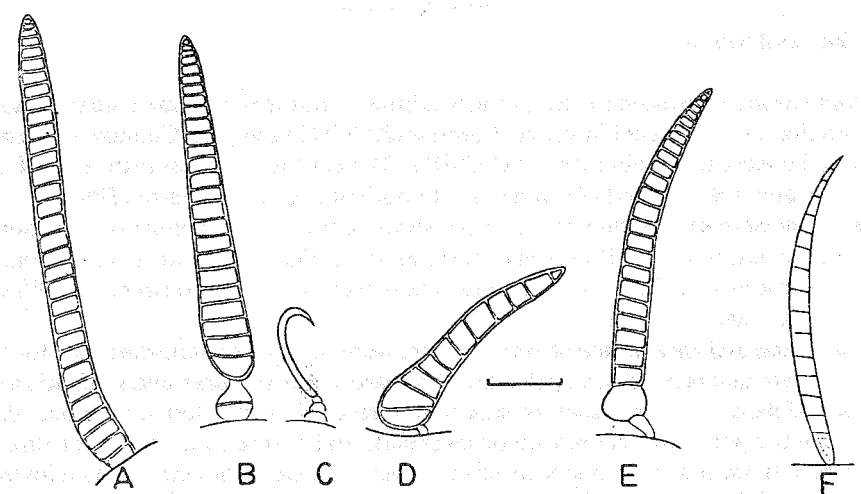

Fig.3. Hairs of different parts of the perianth. A.B. & C. Lip. D. Mouth. E. Trap. F. Prison (scale line = 1 mm).

i) The freshly open flower having a flat perianth lobe (lip) with fresh hairs which are so thick that the opening of the trap is almost closed, the anthers are not dehisced and the stigma is receptive, with expanded lobes. This stage lasts for about 2 h. after anthesis (Fig. 4A & 5A).
ii) The lip starts folding laterally and some of the hairs in the trap start wilting, the anthers remain undehisced and the stigma lobes remain expanded. This stage lasts from 2-24 h. after anthesis.
iii) The lip gets completely folded laterally, the trap becomes open by wilting of the hairs, dehiscence of the anthers takes place and the stigmatic lobes become slightly incurved. This stage lasts from 24-48 h. after anthesis.
iv) The colour of the lip starts to fade, the trap becomes wide open as the hairs of the lip and trap are completely wilted. Pollen grains start germinating within the anthers, trap and prison. Complete inward folding of the stigma lobes with the germinated pollen grains takes place. This starts after 28 h. of flower anthesis (Fig.4 B,C,D & 5B).

Stages (i) & (ii) belong to female or pistillate phase, stage (iii) to male or staminate phase and stage (iv) to post pollination phase.

Type, Structure and Orientation of Hairs: Hairs were observed on the lip, mouth, trap, prison and the stigmatic lobes. There are two types of hairs on the lip: (a) 10 celled hairs, ranging from 364 μm- 406 μm, oriented at right angle to the surface with purple pigment and clear cell wall (Fig.3A,B), (b) 3-4 celled hairs, ranging from 70 μm-84 μm, oriented at right angle to the surface with purple pigment and clear cell wall and having a hook like apical cell (Fig.3C). Only one type of hair is present in the mouth: 10 celled, ranging from 252 μm-280 μm and oriented inward with purple pigment and clear cell wall (Fig.3D). Only one type of hair is present in the trap also: 10 celled, ranging from 392 μm-748 μm and oriented inward, with purple pigment and clear cell wall and having a basal cell and a joint cell (Fig, 3E). Likewise, only one type of hair is present in the prison: 10 celled, transparent, ranging from 308 μm-420 μm, oriented haphazardly and present in patches (but not stellate). These hairs have a purple basal cell (Fig.3F).

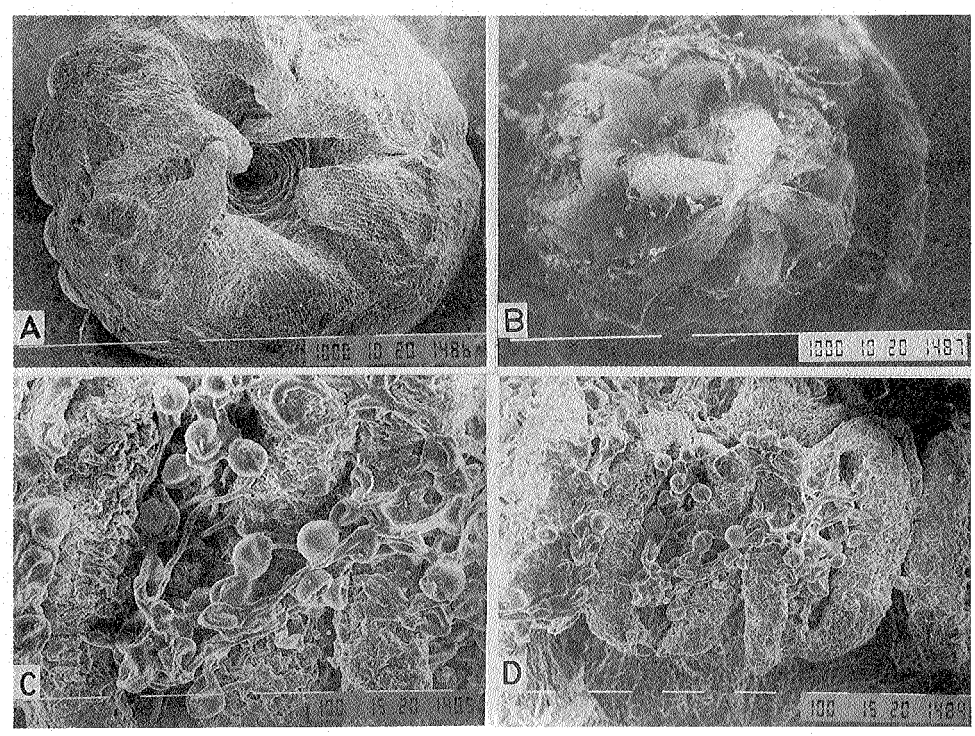

Fig.4. Scanning electron micrographs. A. Receptive (unfolded) stigma lobes of young flower, with wide open stylar canal and undehisced anthers. B. Non-receptive (completely-folded) stigma lobes of old flower, with closed stylarcanal and dehisced an thers. Anthers and stigma lobes with germinated pollen grains. (A.B. Scale bar = 1000 μm). C & D. Insitu, germination of pollen grains (Scale bar = 100 μm).

Stigmatic lobes also have only one type of hairs, which are unicellular, purple, 64 μm-168 μm in length and oriented haphazardly on the acute tip.

Detection of Wax: The test indicates the presence of wax on all parts of the perianth.

Number of Pollen Grains/Flower: The average number of pollen grains per anther is 322 ±2.34 (N = 50), thus total number of pollen grains per flower is 1932.

Odour: Flower emitted a faint unpleaseant smell. The osmophores were found to be present on the mouth (in the form of patches), veins of all perianth parts and on all the lobes of the stigma.

Pollination Efficiency: The flower is pollinated by only a single representative of the genus *Forcipomyia* belonging to family Ceratopogonidae, order Diptera (Fig.5C & D).

(A) ***Number of Insects/Flower:*** The average number of insects per flower is 4.32±1.21 (N = 68), ranging from 1-19 insects per flower.

(B) ***Pollen load/Insect:*** Out of 171 insects collected from 68 flowers, only 10.5% (N = 18) insects carried pollen grains. Each insect carried an average of 16.5±3.23 pollen grains. The distribution of the pollen grains on the body of the insects shows that few pollen grains adhered to the abdomen, wings and legs; the head had the least pollen on it and the maximum number of pollen was found on the thorax (Fig. 5D). Only 4.0% pollen

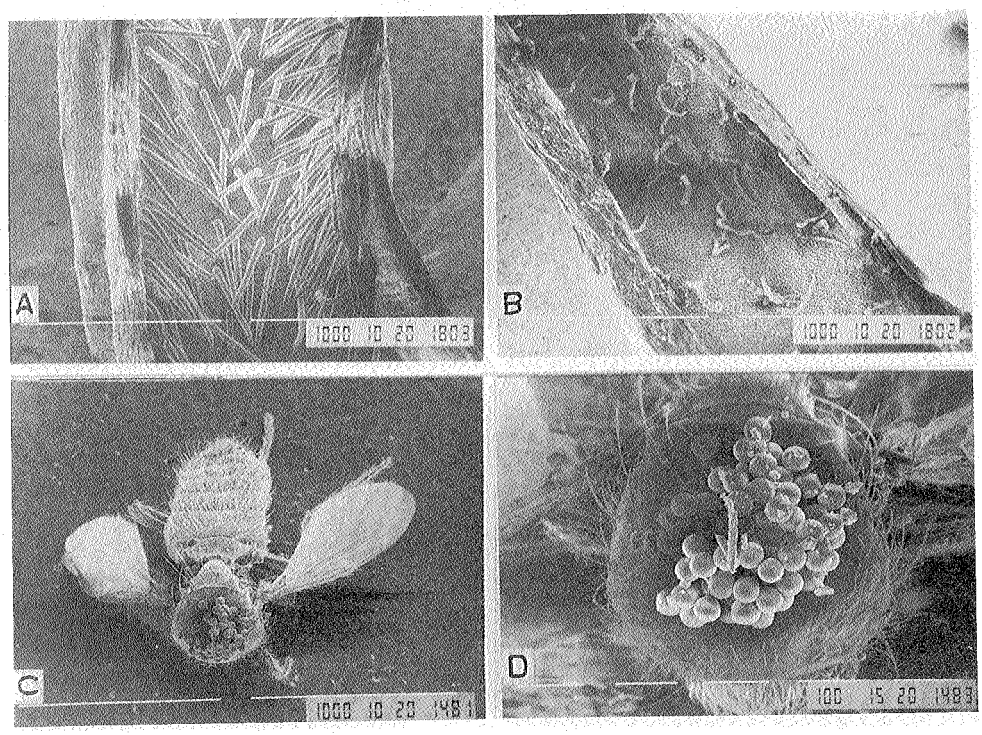

Fig.5. A. young flower with fresh hairs of the trap. B. Old flower with wilted hairs of the trap (A & B. scale bar = 1000 μm). *C. Forcipomyia* sp. (Pollinator) insect (scale bar = 1000 μm). D. Insect thorax with pollen grains (scale bar = 100 μm).

grains (out of total pollen grains produced by the flower) are removed by insects from each flower.

(C) ***Number of Pollen Grains on Stigmatic Lobes:*** : The average number of pollen grains on stigmatic lobes in young flowers (i.e. before anther dehiscence) was 46.14±1.42 (N=31) and in old flowers (i.e. after anther dehiscence) it was 109.4±2.21 (N=3o).

The pollen transfer efficiency (Number of pollen grains on the stigmatic lobes of the flower/total number of pollen grains produced by the flower) in young and old flowers was 2.0% and 6.0% respectively.

Breeding System: Fruits containing normal seeds are formed in bagged as well as in open - pollinated flowers. Two - Way ANOVA revealed significant difference in seed set and seed weight (P) while non - significant difference in fruit set and fruit weight among populations. Significant difference in fruit set, seed set and seed weight (P) while non -significant difference in fruit weight existed between treatments (Table 1).

Discussion

Odour seems to be the primary attraction for the insects. Other attractants like, dark purple colour, shining surface (due to the presence of wax) and the vibration or

Table 1. Results of Two-Way analysis of variance, testing differences between Treatments (Bagged & Unbagged flowers) and Populations.

Parameters	Source of variation	DF	SS	MS	F	P
Fruit Set	Populations	2	0.00188	0.00094	0.26	NS
	Treatments	1	0.01601	0.01601	4.41	<0.20
	Error	2	0.00726	0.00363		
Fruit Weight	Populations	2	0.0177	0.0088	2.53	NS
	Treatments	1	0.0089	0.0089	2.55	NS
	Error	2	0.00699	0.0035		
Seed Set	Populations	2	315.62	157.81	10.38	<0.20
	Treatments	1	235.63	235.63	15.49	<0.20
	Error	2	30.41	15.205		
Seed Weight	Populations	2	0.0000174	0.0000087	1.6	NS
	Treatments	1	0.0002802	0.0002802	13.57	<0.20
	Error	2	0.0000413	0.00002065		

NS= Non-significant.

oscillation of the hairs of the lip, also play significant role in attracting the insects. Only one insect: *Forcipomyia* species (Diptera; Ceratopogonidae) is the pollinator of *A. bracteolata*.

As the insects land on the lip, they may slip inside the trap due to the waxy surface. Similar substances, like grease (*A. lindneri*, Proctor & Yeo, 1973), oil droplets (*A. maculatum*, Meeuse, 1961), tiny granules of wax (*A. sipho*, Meeuse, 1961) are also reported. The trap is lined by the inwardly directed, 10 celled hairs, so that the entry of the insects is facilitated and their exit is barred. Each hair has a small "joint" cell attached to the trap surface on which a big roundish "basal" cell is present. Thus the insects have no trouble in going down, but a return journey is not possible because, as soon as the hairs are pushed upward, the thick basal cells come in contact with the trap wall, making further movement in that direction impossible. At the junction of the trap and the prison, a greenish-yellow coloured ring, devoid of hairs is present (Fig.1). This ring, in view of the transmitted light, appears conspicuous, as compared to the dark prison, and therefore it may guide the insects to wards the prison in view of the fact that many insects have an aversion to darkness and are positively photolactic (Faegri & van der Pijl, 1971).

In the prison, in addition to the greenish-yellow coloured ring, two light windows, with centrally dark portion surrounded by light zones are also present (Fig.1). Generally, the trap is curved towards the window side. Similar windows are reported in *A. sipho* and *A. californica* also. McCann (1943) pointed out that flies in the prison become inactive in the darkness. These windows light the prison and particularly the gynostegium. The windows may also function to draw the insects from the actual outlet (Stebbins, 1974). To the insects the windows appear to be the outlet from the prison so that the insects strike the wall, as they try to come out and in this process brush the stigma.

Like all other fly-trapping taxa, in *A. bracteolata* also, the female phase (pollen receipt) always precedes the male phase (pollen issue). The stigmatic lobes are open and ready to receive the pollen grains which the insects may bring in. The anthers are still closed at this stage. The insects in prison, feed on the nectar, secreted by the nectaries, situated below the anthers and may also feed on the stigmatic excudate. After 24-28 h. of flower anthesis, stigmatic lobes start bending inwards and before complete closing of stigmatic lobes, dehiscence of anthers takes place. The anthers burst explosively and pollen grains reach the stigmatic lobes and spread in the prison and even in the trap also. Insects within the prison are also dusted or loaded with the pollen grains. Most of the pollen grains carried by an insect are deposited on their thorax. After the dehiscence of anthers, wilting of the lip, mouth and trap hairs starts thereby, making it possible for the insects to escape. Insects carry only 4% of the total pollen grains produced by the flower. For about 24-28 h. the insects are imprisoned in the flower. After 28 h. of anthesis, the pollen grains start germinating on the stigma, within the anthers, in the prison and in the trap also. At first the germination of pollen grains starts on the stigma. Stigma received 42% foreign pollen grains of the total pollen grains present on the stigma and 58% indigenous pollen grains, calculated on the basis of number of pollen grains on stigma lobes in young (before anther dehiscence) and in old (after anther dehiscence) flowers. All the pollen grains in all the anthers of a flower germinate 36 h. after anthesis, (Fig. 4C & D), and their pollen tubes reach the stigma and more or less concurrently the pollen tubes enter the style and ovary. Germination of pollen grains within the anthers has also been reported in *Viola odorata* var. *praecox* by Madge (1929) and in *Ottelia alismoides* by Haynes (1987).

A. bracteolata is self-compatible. A significant difference exist in seed set and seed weight while non- significant difference in fruit set and fruit weight among all the 3 populations. Fruit set, seed set, and seed weight varied significantly while difference in fruit weight is non- significant between both the treatments.

The plant possesses a mixed strategy of breeding behaviour. On one hand, complexity of its perianth to trap the insects and protogyny suggest it as an outbreeder and on the other hand, placement and germination of the pollen grains on its own stigma and foemation of fruits, indicate it as an inbreeder.

Acknowledgement

We are indebted to Dr. Laurence A. Mound of British Museum (Natural History) for the identification of insects. We are also grateful to Dr. M. Qaiser for discussion and helpful suggestions.

References

Brantjes, N.B.M. 1980. Flower morphology of *Aristolochia* species and the consequences for pollination. *Acta. Bot. Neerl.*, 29: 212-213.

Cammerloher, H. 1923. Zur Biologie der Blute von *Aristolochia grandiflora* Swartz. *Ost. bot. Z.*, 72: 180-98.

Cammerloher, H. 1933. Die Bestaubungseinrichtungen der Bluten von *Aristolochia lindneri* Berger. *Planta*, 19: 351-65.

Daumann, E. 1971. Zur Bestaubungsokologie von *Aristolochia clematitis* L. *Preslia (Praha)*, 43: 105-111.

Faegri, K. and L. van der Pijl. 1971. *The Principles of Pollination Ecology.* Pergam on Press. Oxford. London. New York.

Graham, R. 1839. *Aristolochia saccata*, Pouch-flowered Birth-wort. *Curtis's bot. Mag.*,, 65: t. 3640.
Haynes, R. R. 1987. Reproductive biology of aquatic weeds (Abst.). *Amer. J. Bot.*, 74: 595.
Johansen, A. D. 1940. *Plant Microtechnique.* McGraw Hill Book Company Inc. New York. London.
Lindner, E. 1928. *Aristolochia lindneri* Berger und ihre Bestaubung durch Fliegen. *Biol. Zbl.*, 48: 93-101.
Madge, M. A. 1929. Spermatogenesis and fertilization in cleistog amous flower of *Viola odorata* var. *praecox Hort. Ann. Bot.*, 43: 545-577.
McCann, C. 1943. Light windows in certain flowers (Asclepiadaceae and Araceae). *J. Bombay. Nat. Hist. Soc.*, 44: 182-184.
Meeuse, B.J.D. 1961. *The Story of Pollination.* Ronald Press Company, New York.
Percival, M. 1965. *Floral Biology.* Pergamon Press. Oxford, Lon don, New York. etc.
Proctor, M. and Yeo, P. 1973. *The Pollination of Flowers.* William Collins Sons & Co. Ltd. Glasgow.
Qaiser, M. 1977. Aristolochiaceae, In E. Nasir and S. I. Ali (Eds.) *Flora of Pakistan.*, 110: 1-4.
Stebbins, G. L. 1974. *Flowering Plant Evolution above the Species Level.* Edward Arnold.
Vogel, S. 1962. Duftdrusen im Dienste der Bestaubung: uber Bau und Funktion der Osmophoren. *Abh. Math.-Naturw. Kl. Akad. Wiss. Mainz.*, 10: 599-763.
Willis, J. C. 1973. A Dictionary of the Flowering Plants and ferns. 8th ed. Cambridge Univ. Press. Cambridge.

(Received for Publication 26 November 1991)