

REGULATED POLLEN ISSUE IN *ISOTOMA*, CAMPANULACEAE, AND EVOLUTION OF SECONDARY POLLEN PRESENTATION

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SUMMARY

The floral characteristics (white, salverform, nocturnal sweet fragrance emission) of *Isotoma petraea* F. Muell. are indicative for pollination by night flying Lepidoptera. The pollen issue is regulated by a valve which has to be operated by the visitor. The valve consists of two connected, dead papillae. The distal parts of these papillae form the trigger hair. A tuft of smaller, also dead papillae under the valve acts as a spring and produces the counter force that repositions the valve after operation. The required force on the trigger hair is 9.5 Dyne. At anthesis the pollen grains are produced inside a closed anther box. Inside this box the piston shaped style gives pressure onto the mass of pollen. On the opening of the valve, this pressure causes the pollen grains to flow out of the box.

A radiant ring of papillae around the style tip acts as a safety valve, that allows the pollen grains to pass if the pressure becomes too high. This happens when the valve is not operated, and the style continues to increase the push forward.

In the genus *Isotoma*, this mechanism of regulated secondary pollen presentation seems to have evolved by differentiation of brush papillae on the anther tips. The evolution in Asteraceae and in Campanulaceae seems to have developed along convergent lines. Firstly secondary pollen presentation produced spatial exactness. Secondly, in at least three independent lines, a temporal exactness evolved, presumably under a selection pressure that acted towards a prolonged pollen protection.

1. INTRODUCTION

Flower biological studies often neglect the distinction between pollen issue and pollen reception, although, as discussed by VAN DER PIJL (1978), these two processes differ fundamentally, even though they often take place in one flower and at the same time. We assume that in many plant species the specific structural adaptations for each process have been mutually incompatible, resulting in a separation of the functions (dicliny, dichogamy). In several other cases, such as the secondary pollen presentation on the style, the structure that promotes the functional precision, participates in both pollen issue and reception.

Structural flower biological adaptations for increase in functional precision evolved independently in many plant taxa, e.g. secondary pollen presentation in Asteraceae, Campanulaceae, Goodeniaceae, Rubiaceae, Proteaceae, Polygalaceae, Stylidiaceae, Fabaceae, Marantaceae, Cannaceae, Myrtaceae. By analysing the structural adaptations, we try to classify these mechanisms and to clarify

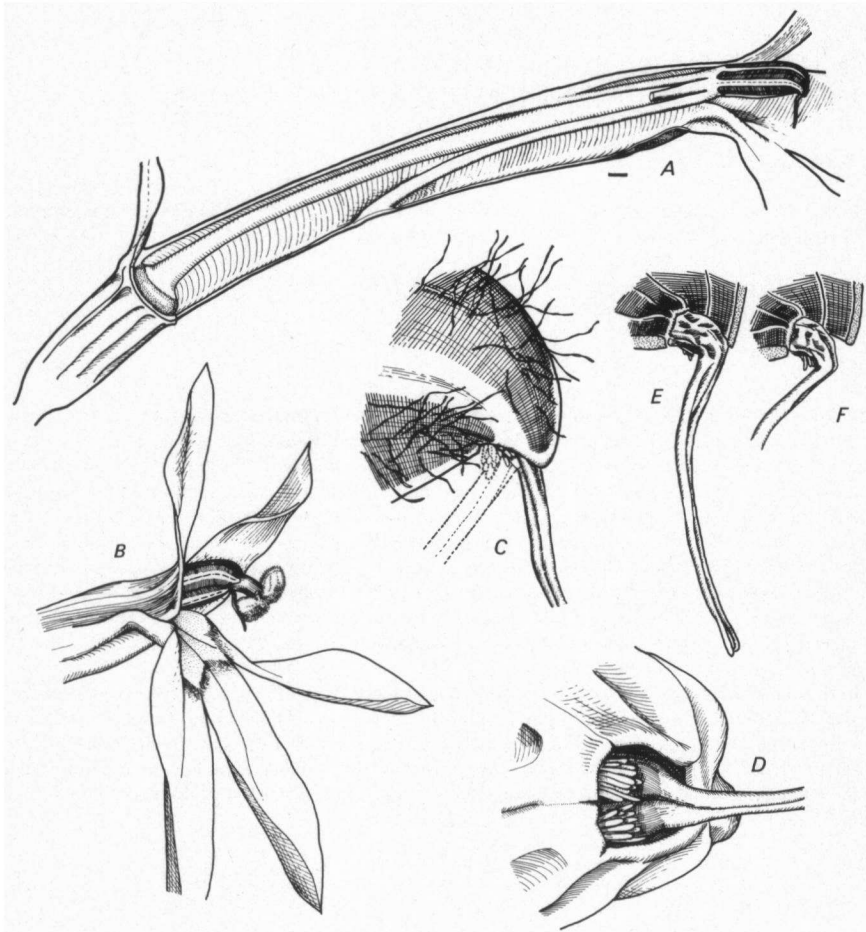


Fig. 1. Flower of *Isotoma petraea*.

A. Longitudinally opened corolla tube of a flower at the start of anthesis.

B. Flower in the receptive phase.

C. Tip of the anther box with trigger hair and spring hairs in the closed position, and in the open position (broken line).

D. Underside of the valve with spring hairs.

E. F. Underside of the opened anther box tip.

E. With the valve in the closed position.

F. With the valve opened.

The bar in A represents 1 mm. B is on the same scale, D, E, F, and G are 8 times enlarged.

the evolutionary forces that produced these fascinating convergences and divergences (BRANTJES & VAN DER PIJL 1980; BRANTJES & DE VOS 1981; BRANTJES 1982b).

In this paper we will analyse in detail the remarkable mechanism that regulates pollen issue in the genus *Isotoma*, (Campanulaceae). This mechanism was described for the first time by MELVILLE (1960). We will discuss its evolution by comparing the regulation of the pollen issue in Campanulaceae with that in related Asteraceae.

2. MATERIALS AND METHODS

Plants of *Isotoma petraea* F. Muell. were grown in the hot green houses of Hortus de Wolf, Rijks Universiteit Groningen. For description of phenology 22 flower-buds were individually tagged and their flowering was observed during the nights from 21 July till 5 August 1980. Drawings were made with a camera lucida mounted on a dissecting microscope. Anatomical sections were made after fixation of flowers during several days in FAA (2% formalin, 5% acetic acid, 65% ethanol), and embedding in paraplast. The forces necessary for operating the flower valve were measured with a torsion based force meter (BRANTJES 1981a).

3. RESULTS

3.1. The flower

The white salverform corolla consists of a long narrow tube, and 5 subequal lobes (*fig. 1, a, b, c; fig. 2*). Short, longitudinal folds divide the tube entrance into an upper and a lower opening (*fig. 2, b*). The anther box fits in the upper opening and is kept in position by the folds. The narrow lower opening is accentuated on the 3 lower petal lobes, by a greenish star (*fig. 1, b; 2, a, b*) bordered with a purple band; a mark which might act as a nectar guide. A nectary disk, near the base of the corolla tube, produces a large quantity of nectar. The filaments are partly continuous with the corolla (*fig. 1, a*). The filament tips fuse with each other and their theca form a hard and closed box, in which the pollen is released. The pistil lies freely in the corolla tube, ending in the anther-box.

3.2. Phenology

The flower opens on its first blooming day, between 19.^h00 and 21.^h00, and thereafter does not close until wilting. Only during night the flower emits a distinct sweet odour, which resembles the nocturnal fragrance of the phalaenophilic *Melandrium album* (Mill.) Garcke (BRANTJES 1976). Flower development passes through two distinct phases. Pollen issue during the first three of four nights (*fig. 3*): phase one. In the second phase the stigma lobes spread and are receptive (*fig. 1, b*).

3.3 Pollinators

The pollinator type can easily be deduced from the floral characteristics. The salverform corolla, with deep hidden nectar – accessible only through a restricted entrance – are indicative for Lepidoptera as pollinators. The anthesis in the evening, the exclusive nocturnal and sweet fragrance, together with the white, star-



Fig. 2. Flower in the pollen issue phase; lateral view (A); and frontal view, before (B) and after (C) operation.

shaped corolla, point at nocturnal Lepidoptera (cf. FAEGRI & VAN DER PIJL 1979). A kind of foothold provided by the lower three lobes suggests phalaenophilic pollination, although this does not exclude sphingophily. The very long and weak pedicel, bending under the slightest touch, allows for little weight on the flower. The moths will therefore have to remain at least partially in a hovering position. A comparable pollination mechanism was recorded by VOGEL (1954) for *Isotoma longiflora* (sphingophily) and for *Isotoma axillaris* (pycho-phily).

3.4. The pollen presentation mechanism

Together, the connected thecae constitute a box, in which their pollen is shed. There is a very small opening facing downwards at the end of the box, which is closed by a valve (fig. 1, d, e, f; 4, a). Anatomical sections demonstrate that the valve consists of the basal portions of two giant cells. The upper parts of these cells form the trigger hair. Forces exerted on the trigger hair cause the valve to hinge in the middle of the basal part (dots in fig. 5, a). Thus the hinge is not situated on the connection with the theca. On the under side of the valve, the theca bears two tufts of unicellular stiff (spring-)papillae (fig. 1, d). Upon opening, the hairs in these tufts become deformed, thus producing a force to return the valve to its original closed position. Anatomical sections of the flowers at anthesis revealed that both the trigger-hair cells and the spring-papillae are

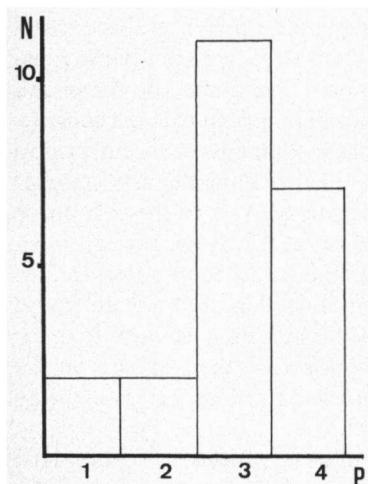


Fig. 3. Duration of the pollen issue phase of 22 flowers.

N, number of flowers.

P, nights with pollen issue.

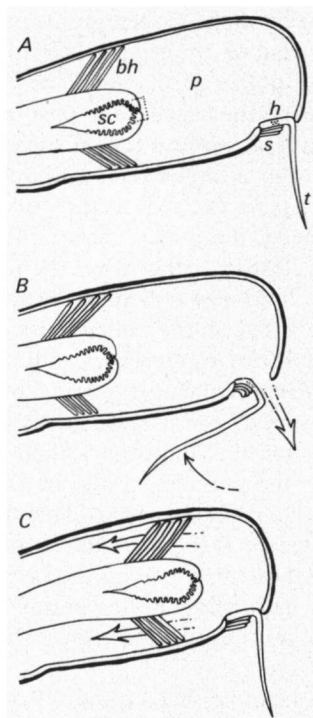


Fig. 4. Schematic longitudinal section through the anther box.

A. At begin of anthesis.

B. after a push on the trigger the valve opens and pollen grains come out.

C. Later during anthesis, in case the valve has not been opened, part of the pollen grains pass between the brush hairs.

empty and, therefore, dead. Therefore, stiffness and repositioning of the valve must be passive mechanical processes. Turgor, which is often a mechanism in movable plant parts, does not play any role here. To test the mechanical aspect, several theca boxes were taken from the flowers and dried for several days. Thereafter, it was found that the valve-hinge still opened and closed, similar to the processes in the intact flowers.

During the first flowering phase the style penetrates into the theca box at its base. The two stigma lobes are folded together, enclosing a free space. The interdigitation of papillae, where the edges of opposite lobes touch each other, firmly close the inter-lobe space (fig. 5). In this way the inner sides of the lobes remain well protected from contamination by own-pollen under the pressure inside the anther box. On the outside of the style top is a ring of brush-papillae, that fits in the theca box like a piston in a tube.

During night, pollen grains never leave the anther box spontaneously. Only after pushing the trigger hair towards the flower-base, the opening releases a flow of pollen grains (*fig. 2, b; 4, b*) which flow after several seconds will cease. Releasing the trigger, and pushing a second time can sometimes produce a new pollen flow. After a few of such operations no more pollen grains are released until after a longer pause (about 15 minutes). The explanation for this lies in the pressure exerted on the pollen mass inside the theca box. When the valve is opened, the pollen mass flows out and the pressure inside the box relaxes. After closure of the valve, the position of the remaining pollen grains is rearranged and new pollen grains accumulate over the valve. Apparently the pressure exerted on the pollen mass inside the theca box is generated by the piston (the style tip), by growing continuously into the box. This pressure is not generated at the moment of triggering, by a shortening of the filaments or by elongation of the style, as was verified by painting marks on the filaments and style. It was found that these marks had not moved during triggering. In the first phase flower the pressure inside the box never reaches a level at which it can open the valve from the inside. This finds an explanation in the observation that in untriggered flowers, in the second and third night, pollen grains were found in great quantity behind the brush hairs of the style tip. This indicates that the brush hairs allow pollen grains to pass the piston in case the pressure on the grains exceeds a certain level (*fig. 4, c*). The brush hairs then act as a safety valve.

It was found that a force of 9.5 Dyne (number of observations = 14, standard deviation = 2.6) exerted near the top of the trigger hair completely opened the valve. This is the smallest force observed till now for operation of flowers with movable parts (BRANTJES 1981c). In most flower-pollinator combinations the required force is related to the weight of the pollinators (BRANTJES 1981b, c, d 1982a) and works as a mechanism for selection of the pollinators. Here, the force is not in relation to the expected weight of the pollinators (expected to be in the range of between 0.6 and 6.0 g). The trigger force therefore, will not assist in selection of pollinators by power. This is presumably because, if the trigger would require too much force, it would prevent the moths from advancing their heads until under the pollen discharging opening. Of course the plant species possesses other mechanisms for selection of pollinators, e.g. by the flower-tube length.

Finally, in the second phase flower, on the third or fourth day, the pistil breaks through the top of the theca box, and some pollen grains fall out. The stigma lobes then spread and are receptive for many days.

4. DISCUSSION

As pointed out by MELVILLE (1960) all Australian species of *Isotoma* possess a similar trigger hair mechanism. *I. longiflora* (L.) Presl. and several other species placed by some authors into the genus, lack this trigger hair mechanism and could, therefore, better be excluded from *Isotoma* (MELVILLE 1960). This is an

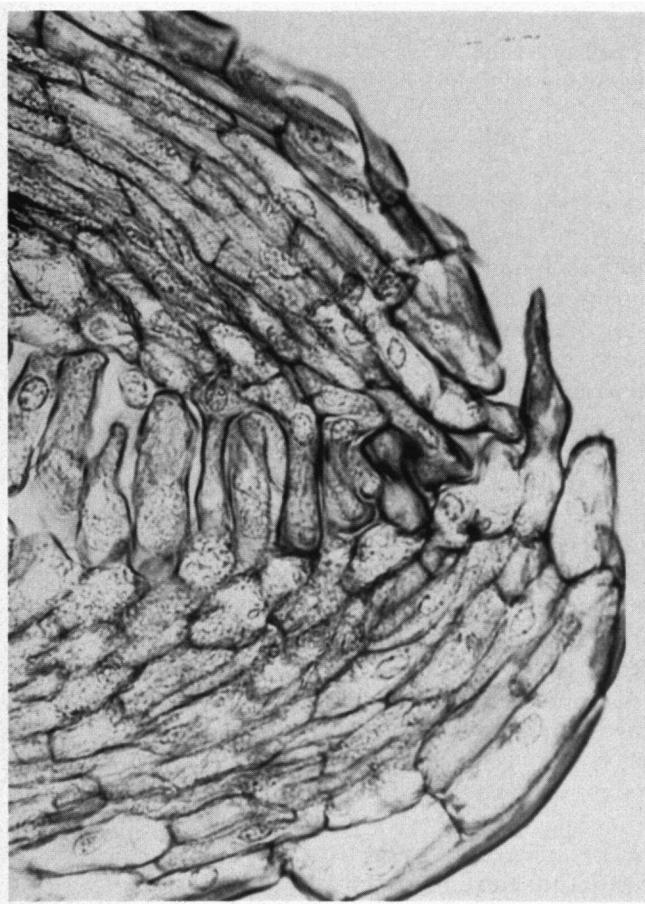


Fig. 5. Zip-closing by the rims of the stigma lobes.

The position of this detail is indicated in fig. 4, A, by a dotted line.

P = mass of pollen grains.

BH = brush hairs.

SC = stigmatic cavity.

H = hinge in the valve (dotted area).

S = spring hairs.

T = trigger hair.

example of the use of a flower biological precision mechanism for taxonomic delimitation.

In *I. longiflora* the mouth of the theca box bears tufts of papillae, which act as a brush that sheds the pollen grains over the visitor as it touches the anther tube. From such a brush, a fine regulated mechanism might have evolved by the differentiation into two types: the valve and the spring papillae.

Also the flowers in the distant genus *Lysipomia* (Campanulaceae) seem to possess differentiated papillae on the two lower thecae (MELVILLE 1960). This might represent parallel evolution from undifferentiated brush hairs or both genera might share a common ancestor with trigger hairs.

A convergent evolution apparently occurred in *Thunbergia grandiflora* (Acanthaceae), where pollen is arrested until the pollinator touches a trigger hair on the anthers (FAEGRI & VAN DER PIJL 1979).

The sophisticated pollen presentation mechanism in *Isotoma* shares many functional aspects with those found in some of the Asteraceae, as will be discussed here (fig. 6). Similar to both families, and presumably already present in their common ancestor, are: 1) pentamerous androecium, calyx and corolla, 2) tubular corolla, 3) nectar disk on top of an inferior ovary. The multiovulate gynoeceum in Campanulaceae contrasts with the monovuly in Asteraceae. The compensation, by flower aggregation into capituli enlarged the number of ovules in the "blossom".

In the Campanulaceae two main lines of pollen presentation in flower buds exist. In the Campanuloidae, the anthers transfer their pollen onto the outside of the style, in an area of specialised papillae. From these, the pollen becomes smeared onto the vector. In the Lobelioidae, the anthers become interconnected like in Asteraceae, and the style pushes the pollen out, onto the vector.

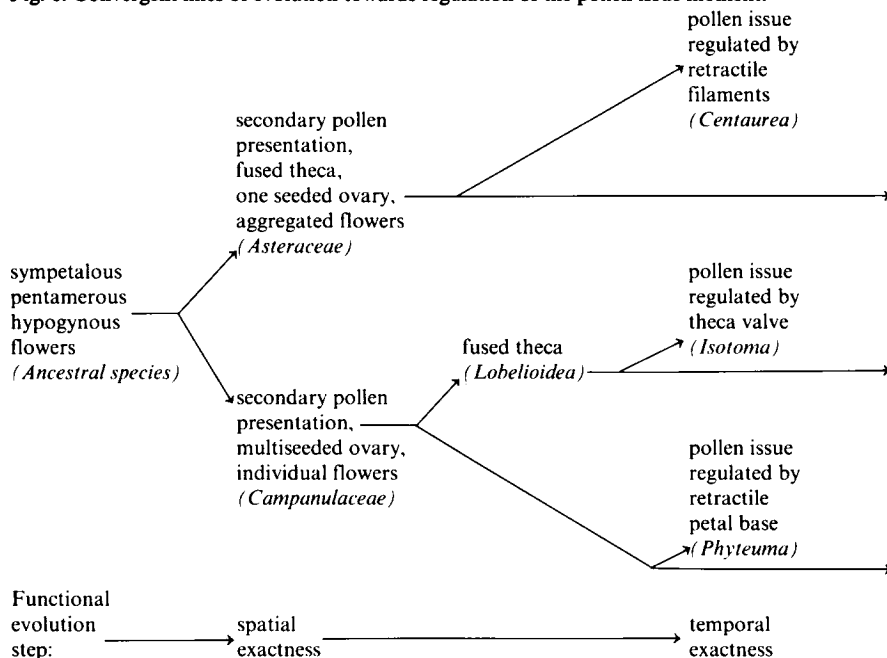
One selective advantage that might be held responsible for the evolution of secondary pollen presentation on the style, is the high exactness that can be reached in the pollen issue and reception. Both events can happen in exactly the same spot in the flower, and, provided that the pollinator takes a constant position in each flower, only a very small spot on the pollinator will receive pollen and touch the receptive stigmas. The resulting effects (selective advantages) can be:

a. The exactness increases pollen economy, by reducing waste of pollen, which otherwise would become smeared on not appropriate body parts.

b. Reproductive isolation can be realised, even by simultaneously flowering sympatric congeneric species with identical pollinators. This occurs e.g. in *Polygala* spp. (BRANTJES 1982b). These specific advantages of the secondary pollen presentation mechanism became partially irrelevant in the Asteraceae by their monovuly and the aggregation of florets into flowerheads which is regarded as an adaptation to venter pollination by bees (FAEGRI & VAN DER PIJL 1979).

Another secondary advantage that might have made these Asteraceae to conserve the secondary pollen presentation mechanism can be in the prolonged protection after anther dehiscence, of the pollen in the tubular androecium. This protection could be against adverse climatic factors (humidity, desiccation radiation such as solar UV), as well as against non-pollinating pollen gatherers. The short viability of Asteraceae pollen is well known to pollen-physiologists (a.o. HOEKSTRA 1979). One might, however, question whether this short viability is evolutionarily a consequence of the extended protection, or is a selective force for its evolution. The protection ends when the pistil drives out the pollen. Many Asteraceae evolved a mechanism to delay this issue until the pollinator arrives

Fig. 6. Convergent lines of evolution towards regulation of the pollen issue moment.



on the flower, e.g. *Centaurea* with its sensitive and retractile filaments (FAEGRI & VAN DER PIJL 1979).

A similar prolongation of protection with sensitivity to stimulation, can also be found in *Phyteuma* (Campanulaceae), where the style with its adhering pollen remains in a tube formed by the interconnected petal tips. This style becomes exposed after a triggered retraction of the petal basis. It might be called secondary pollen protection in combination with secondary pollen presentation. Also in *Isotoma* (Lobelioidea) a mechanism for the prolonged protection has evolved. In contrast to the mechanism of the Asteraceae and of *Phyteuma*, the *Isotoma* mechanism consists of dead cells, and reacts passively to forces.

Pollinators (Lepidoptera) get a maximum pollen load in a more or less exact and safe spot, i.e. on the head where it will be difficult to be reached by the cleaning movements of the legs. This same spot receives intensive contact with the stigma of receptive flowers.

Together, to the *Isotoma* mechanism can be attributed several advantages: 1. Maximum duration of the protection of pollen against climatic factors. 2. All kinds of pollen collecting insects cannot reach the pollen, which means a reduction in the pollen loss. 3. Spatial exactness in deposition onto and reception from the vector of the pollen.

These potentials might explain the high number of ovula in each *Isotoma* flower.

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