

Pollen Placement and Reproductive Isolation Between Two Brazilian *Polygala* Species (*Polygalaceae*)

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(Received November 12, 1981)

Key Words: *Polygalaceae*, *Polygala vauthieri*, *P. monticola* var. *brizoides*. —Reproductive isolation, pollination ecology, secondary pollen presentation, stigma secretion, bee pollination, pollen protection.

Abstract: *Polygala vauthieri* and *P. monticola* var. *brizoides* have secondary pollen presentation from a basket-like structure on the style apex. This basket is loaded after the first visit by a bee. Pollen reception, therefore, can precede the issue of pollen. A sticky stigma secretion glues the pollen from the basket under the head of the bee visitor in an exactly predetermined spot on the left side only. This position mostly forms a kind of safe spot, where the bee can not remove the pollen. The exact position on the bee's head is determined by the species specific distance between style tip and nectary in the visited flower. In this way the two sympatric species deposit the pollen 2 mm apart on the visitor and so can avoid hybridization pollination, while being visited by the same group of bees.

The flowers of the genus *Polygala* seem to have a uniform general construction, with only minor modifications on the basic family pattern. At the same time the flowers seem highly specialised, with zygomorphy, petal-like sepals, three peculiar petals and secondary pollen-presentation on the style apex. Information on pollination in this genus as well as the other genera of the family *Polygalaceae* is very scarce (HILDEBRAND 1867, MÜLLER 1875, MACLEOD 1891, SCOTT-ELLIOT 1891, DUCKE 1901–1902, KNUTH 1904, SCHOENICHEN 1922, GOEBEL 1933, VOGEL 1954, FAEGRI & VAN DER PIJL 1979). Therefore, it was decided to study the reproduction of representatives of this genus in Brazil. In this article we report on the pollination of *Polygala vauthieri* CHOD. and *P. monticola* H. B. K. var. *brizoides* (ST. HIL. & MOQ.) STEYERM. (= syn.: *P. angustifolia* KUNTH), both in the Section *Hebeclada* CHOD. A discussion

about the pollination of other species in the family and observations on seed dispersal in this family will be communicated elsewhere (BRANTJES & VAN DER PIJL 1980, and BRANTJES in prep.).

Materials and Methods

The observations were made in Botucatu, State of São Paulo, Brazil, on 17, 18 February, 7, 18, 21 March 1978. The plants grew in ruderal vegetation of grass mixed with herbs, situated between a forest edge and the side of a path leading to the chapel of Rubião Junio, 885 m above sea level. The position of pollen on the insects was determined with a low magnification microscope. Drawings were made with camera lucida. Voucher specimens have been deposited in the herbaria of the Universities of Botucatu (S.P.) Brazil, Campinas (S.P.), Brazil, and Utrecht, The Netherlands. The insects are in the Rijksmuseum voor Natuurlijke Historie, Leiden, The Netherlands, collection numbers 70240101 to 70240108 and 70250101 to 70250116.

Results

Description of the Flower of *Polygala vauthieri*. From 8.30 to 9.00 a. m., during anthesis the lateral sepals spread. The odourless flowers remain open during the whole day. All petals and the lateral sepals are blue. The two upper petals have an upturned apex, with dark-blue longitudinal stripes (Fig. 1). In the lower petal three parts can be distinguished (Fig. 2*a*). The basal part, 2 mm in length, is free from the upper petals and the anthers. The second part, the carina, is formed by an inflated cup which hinges on to the first, basal part. The lateral margins of the carina touch each other above. Transverse folds form ridges in the lateral margins. At the basal end these margins end in a blunt tooth underneath the upper petals; the tooth is directed towards the flower base. Longitudinal folds separate the yellow-green third part. The two sides of this apical part are folded together. On top it bears two triangular lobes. This third part of the petal is always positioned slightly to the right of the flower, so the flower is somewhat asymmetrical (Fig. 1). The stamens are connate in two bundles of four filaments each. The ovary stands on a large nectary disc (Fig. 2*b*). The swollen and stiff basal part of the style inclines downward. The thin distal part of the style bends up sharply. In the flower the bend is situated just in front of the petal hinge. At the top of the style is a pollen basket formed by unicellular hairs (Fig. 2*c*). The anthers deposit the pollen grains into this basket (secondary pollen presentation). The stigma is apical to this basket. At anthesis the stigma secretes a large amount of sticky fluid when touched. In the flower the style apex, with the pollen basket and the stigma stands between the apical lobes of the lower petal.



Fig. 1. *a* Flowers of *Polygala vauthieri*; — *b* *Apis africana* hybrid visits *P. monticola* var. *brizoides*; — *c* *Exomalopsis* sp. 2 (70250105) visits *P. monticola* var. *brizoides*

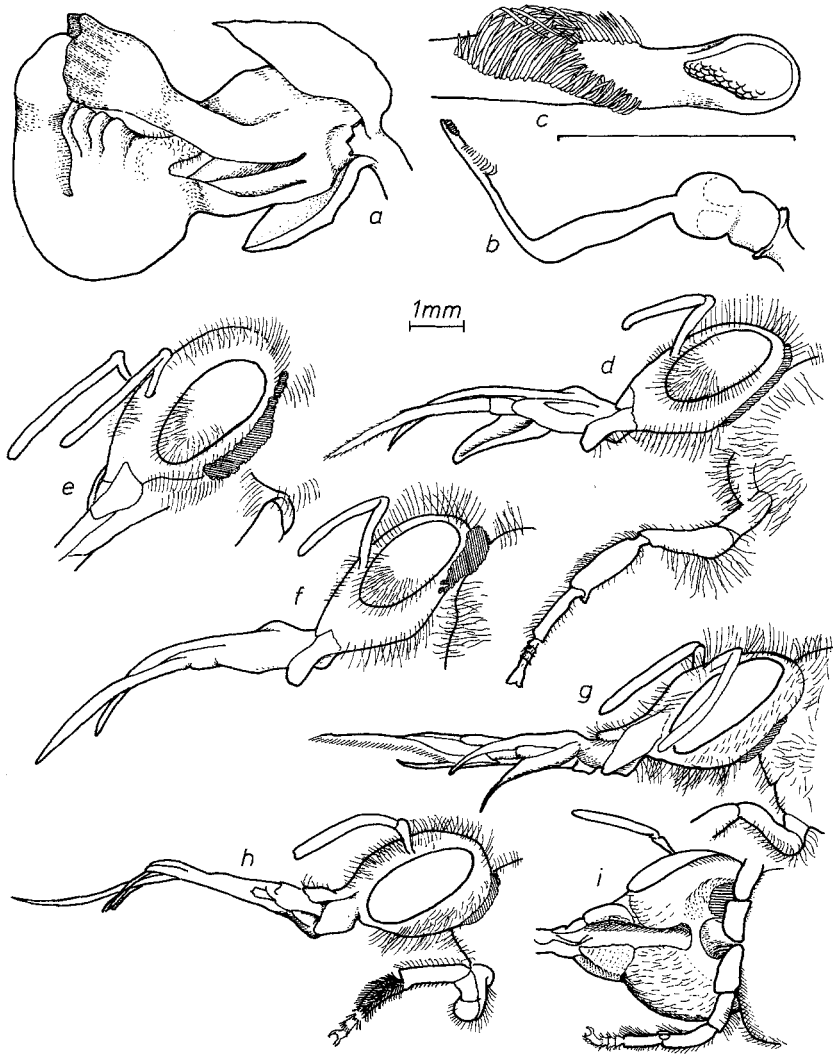


Fig. 2. *Polygala vauthieri* CHOD. and visitors. *a* Lateral aspect of the flower, after removal of the lateral sepal; — *b* isolated style; — *c* detail of the style tip; — *d, e, f* *Apis africana* hybrid (70240101, -02, -05); — *g* *Megachile* sp. (70240107); — *h, i* *Hypanthidium* sp. (70240104). — *d, e, f, g, h, i*: the drawings are from fresh specimens. All drawings, with the exception of *c*, are at the same magnification, see bar which indicates 1 mm

The Pollination Process. For pollination the carina of the lower petal must be bent down and the visitor must touch the style tip. Indeed, a slight pressure was sufficient to bend down the carina. At the same time the style slipped between the apical triangular lobes and became exposed. After release, the carina sprang back to its original position and hid the style again. This mechanism could be operated several times. In the morning, in ten unvisited flowers, the first artificial bending of the carina revealed a large stigma-gland which was still free from pollen grains. Always the basket under it was half filled with pollen grains. After letting the carina spring back and operating the mechanism a second time, pollen appeared on the stigma and the basket contained more pollen grains than before. The biggest pollen load was usually in the right side of the basket. Clearly, in the first operation the apical carina fold retained a certain amount of pollen, which the style tip scraped away during the second operation. The observation indicated that at the time of the first insect visit the stigma will be free from pollen, and can therefore pick up foreign pollen. At the same time the viscous substance can be smeared onto visitor and fresh pollen, from the basket, can stick to it. After the first visit the style tip is reloaded with additional pollen from the same flower. In this way pollen reception precedes pollen issue. Here, this is a requisite for cross-pollination, because, after the first visit, large masses of the flower's own pollen adhere firmly in the viscous substance on the stigma, and form a big clump that will inhibit later-arriving foreign pollen.

The Visitors of *Polygala vauthieri*. On the basis of their behaviour the visitors fall into two groups: those that operate the hinge and those that do not. Of the latter, small bees (cf. *Ceratina* sp.) landed on top of the upper petals and inserted the proboscis on the side of the flower between the upper and lower petals. On landing, *Melissodes* sp. (*Eucerini*) (coll. nr. 70240103, 70240106), both males here, grasped the upper and the lower petals together to hang obliquely in front of the flower. They also inserted the proboscis through the space between the two petals, always on the left side of the flowers. Because all these bees did not operate the hinged carina, they did not contact the essential parts of the flower, and did not pollinate it. The two male *Melissodes* sp. bees were examined, but no pollen was detected on their bodies.

The other group of visitors, *Megachile* sp. (*Megachilidae*) (70240107) and *Apis africana* hybrid (70240101, 02, 05, 08), all grasped several petals and sepals together, to hang in front of the carina. The carina always bent fully down and back, before the bee probed under the upper petal with the proboscis. It looked as if the bees tore the carina

Table 1. Position of the pollen on the visitor

Visitor (collection number)	number of observations	Position of the pollen	Distance from the tip of the tongue in mm*
On: <i>Polygala monticola</i> HBK var. <i>brizoides</i> (st. HIL. & MOQ.) STEYERM.			
<i>Apis africana</i> hybrid (70250109)	7	on inner side of mandible under the eye	6-7
<i>Exomalopsis</i> sp. 1 (70250106)	1	back of head and on coxa of front leg	5-6
<i>Exomalopsis</i> sp. 2 (70250105)	1	under the eye	5
<i>Megachile</i> sp. (70250107)	1	under the mandible	6
<i>Coelioxys</i> sp. (70250108)	1	under the mandible	6
On: <i>Polygala vauthieri</i> CHOD.			
<i>Apis africana</i> hybrid (70240101, -02, -05)	3	back of head, gena	8-9
<i>Megachile</i> sp. (70240107)	1	back of head, occiput	8-9
<i>Hypanthidium</i> sp. (70240104)	1	back of head, occiput	8-9

* The distance was measured in freshly killed specimens, of which the proboscis was pulled out between the mandibles, and was held in that position by closing the mandibles behind the proboscis.

In this way the proboscis will be stretched more than occurs in the live insect during drinking.

down, to remove this obstacle on the way to the nectar. The lateral ridges on the edges of the inflated carina presumably provided a good foothold. When the bee left the flower, the carina bounced back. Each visit lasted one to two seconds. Flowers visited earlier by other bees were frequently visited again.

The bees that were collected all had a clump of pollen grains in a sticky mass on the back of their head (on the genal area or the occiput mostly) (Figs. 2*d-i*, Table 1). The pollen mass was always located on one definite spot, on the left-side of the head, 8 mm from the tip of the

proboscis. This constant distance corresponds with the distance between the style apex and the nectary. Therefore, when the bee drinks from the flower, the stigma touches the bee's head on this spot, depositing the viscous substance, to which the pollen can stick. The location, on the left side only, corresponds with the slight asymmetry of the carina apex. The inward orientation of the stigma and the basket corresponds with the contact point on the bee: the genal area or the occiput. This position seems functional, as it will not easily be reached by the legs when the bees groom pollen from the hairs. In the corbiculae of two *Apis* visitors were found both Compositae pollen and Polygalaceae pollen. The last might have been collected directly from the flowers, or from the excess on the head, where both specimens had a large pollen deposit. A similar deposition of pollen in a safe place on the head, out of reach of the bee, also occurs in *Calathea* (*Maranthaceae*) (KENNEDY 1978).

The Flower of *Polygala monticola* var. *brizoides*. At about noon the lateral sepals close. The general flower structure in *P. monticola* var. *brizoides* (Fig. 1*b, c, 3*), is similar to that of *P. vauthieri*. The most obvious differences are:

a) the dimensions: the flower of *P. monticola* var. *brizoides* is a little smaller than that of *P. vauthieri*;

b) the basal part of the lower petal is continuous with the staminal tube and with the upper petals, and therefore, the hinge appears to be attached on the underside of the staminal tube (Fig. 3*b*);

c) the apical asymmetry of the carina is more pronounced in *P. monticola* var. *brizoides* than in *P. vauthieri* (Fig. 3*a*);

d) the style is stiff along the full length (Fig. 3*d*) with its bend 2 mm in front of the hinge;

e) the style apex: on the left of the style apex, under the viscous stigma stand two combs of hairs that form a pollen basket (Fig. 2*e*). This asymmetry of the style is attained by a torsion through an angle of 90° in the top part (Fig. 2*c*). In the flowerbud, one day before opening this torsion is only 45° (Fig. 3*d*) and the style is shorter than that in the open flower. In younger buds the torsion is absent. Therefore, the torsion may originate from differential growth.

When the petal cup is first lowered, in *P. monticola* var. *brizoides* as in *P. vauthieri*, the stigma is free from pollen grains. On repeating the operation the mass of pollen grains in the basket and on the stigma increases (Fig. 3*c*).

The Visitors of *Polygala monticola* var. *brizoides*. Insects only visited the flowers until about noon. *Hesperiidae* (*Lepidoptera*) landed on the

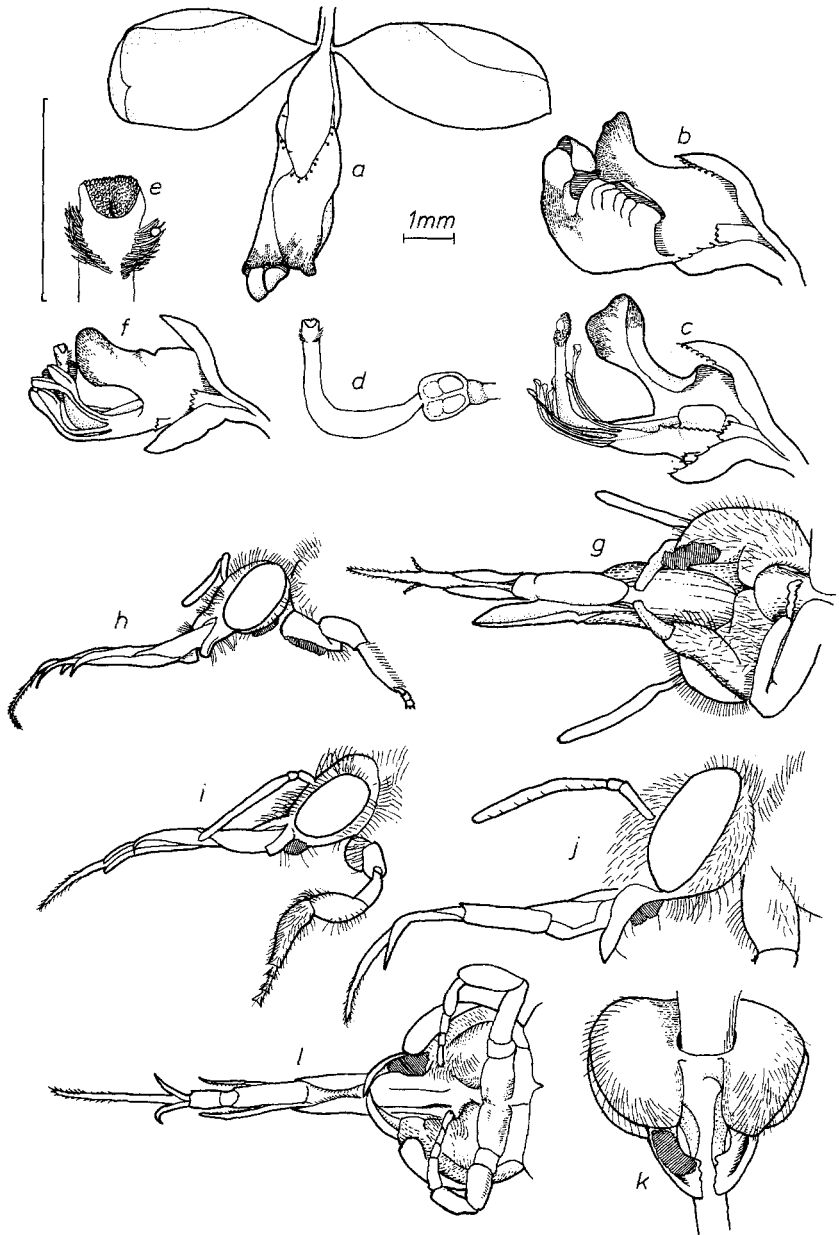


Fig. 3. *Polygala monticola* var. *brizoides* and visitors. *a* upper aspect of the flower; — *b* side view of the flower after removal of the lateral sepal; — *c* side view of the flower after removal of the lateral sepal, the lower petal and the left-hand upper petal; *d* isolated style; *e* petal of the style tip; — *f* side view of the flowerbud one day before anthesis; the lateral sepal and the lower petal have been removed; — *g* *Apis africana* hybrid (70250109); — *h* *Exomalopsis* sp. 1 (70250106); — *i* *Exomalopsis* sp. 2 (70250105); — *j, k*: *Megachile* sp. (70250107); — *l* *Coelioxys* sp. (70250108). — *g, h, i, j, k, l*: the drawings are from fresh specimens. All drawings, with the exception of *e*, are at the same magnification, see bar, which indicates 1 mm

flowers and became hung below the corolla tube. In this position, the proboscis was always inserted from the side, between the upper and the lower petal. The carina was never pulled down. On the body of three *Hesperiidae* specimens, no pollen grains were detectable. One small bee (cf. *Ceratina* sp.) sat on top of the petals and inserted the proboscis from the left between the petals. Such visitors will not pollinate. All other visitors (Table 1) (Fig. 1*b, c*), operated the hinge mechanism of the carina, before they inserted the proboscis, in the same way as visitors on *P. vauthieri* did. Similarly the pollen deposits on the bees (Table 1) were on the left side of the insects, and here at a distance of 6 mm from the proboscis tip (Fig. 3*g-k*). This distance corresponded with the dimension of the flower (stigma-nectary). The size of the insect seemed to determine on which body part the pollen grains were deposited (mandible, head, coxa). The asymmetry of the lower petal and the orientation of the stigma correspond with this unilateral pollen deposition.

Reproductive Isolation. *Polygala vauthieri* and *P. monticola* var. *brizoides* were growing together at the study sites. Both species seemed to attract a wide spectrum of small bees. Even if there is differential attraction, based on flower size (e.g. *Exomalopsis* sp. 1 (70250106) might be too small for *P. vauthieri*), it is very probable that several of these bees will visit both *Polygala* species: e.g. *Apis africana* hybrid. and *Megachile* sp. were collected from both species. However, hybridizing pollination will not occur because of the precise mechanism of pollen deposition and reception in these *Polygala* species. The location of the pollen clumps deposited by the two species on their visitors do not overlap. This precise localisation of pollen on the vector as a means of reproductive isolation is reminiscent of that found in the *Orchidaceae*.

Additional reproductive isolation can result from differences in flowering time in the day: flowers of *P. monticola* var. *brizoides* were open and visited only during the morning hours, and *P. vauthieri* during the whole day. Consequently in the afternoon there is no possibility of mixed visits by the insects.

Discussion

The morphology of the flowers of *Polygala* suggests precision pollination. Observations on the pollinating processes confirm this. The precision of pollen deposition and reception allows the plant to reduce the number of pollen-grains produced per flower. This reduction can be

reinforced by the protection offered by the carina which envelops the style. In this way pollen-eating flies and other insects are excluded, so that pollen wastage is reduced. Illegitimate and legitimate visitors were seen only to drink nectar. Pollen was almost not collected actively. This may partly be because the quantity is insufficient to allow pollen-collecting bees to build up a search image. The other part of the explanation can be in the location of the pollen on the insect. All pollen was deposited on the underside of the head or on the back of it. This place is difficult to reach in grooming: again this prevents loss of pollen. Because, for the plant pollen represents a substantial investment of protein-nitrogen and other minerals, economy in pollen usage will be a selective advantage for the plant.

In *P. monticola* var. *brizoides* and *P. vauthieri* pollen deposition shows a precision which is recorded here for the first time in *Polygala* species. This effects reproductive isolation, although both species have a wide spectrum of visiting bees and share the same pollinator species.

Another aspect of this very precise mechanism is the development of asymmetry, which results in the deposition of the pollen on the underside or on the back of the head. In this transference process, the median proboscis is in the way of the projecting style, which better can reach the head from one side, the left side of the pollinator. This presumably also applies to the asymmetric flowers of the African *P. bracteolata*, which, however, works on the other side, the right side of the pollinators (SCOTT-ELLIOT 1891). In each *Polygala* species there are only left-handed or only right-handed flowers. Therefore, there is no enantiomorphy, which means that two types are present in the same species.

Unless the flower has special means for prevention of selfing, pollen presentation on the style apex will result in self-pollination. The adverse effects of this cannot be overcome with a genetical system of self-incompatibility alone, because the own pollen will reach the stigma in such large quantities that it may hinder the adherence or the germination of foreign pollen. VAN DER PIJL (1978) discussed similar cases of stigma contamination. The preventive measure that seems most common in plants with secondary pollen presentation is a marked proterandy (*Asteraceae*, *Goodeniaceae*, *Campanulaceae*, *Protaceae*). The other way out of the danger can be very high precision in the location of pollen deposition on the style, together with a mechanism to ensure that the stigma is effectively pollinated on the first visit. *Polygala monticola* var. *brizoides* and *P. vauthieri* show such a mechanism as also do *Maranthaceae*, where, in one movement, the stigma scrapes off the pollen from the vector prior to deposition of new pollen (KENNEDY

1978). "Functional protogyny" seems to be an inadequate term for this process because pollen presentation and reception happen almost at the same moment.

Another parallel between *Polygala* and the *Maranthaceae* is in the production by the style of a sticky substance (see also SCHOENICHEN 1922, GOEBEL 1923) which helps the pollen-grains to adhere to the pollinator, as also in *Vinca* (*Apocynaceae*). Also in *Orchidaceae* the stigma (rostellum) produces the adhesive for the pollinia.

Polygala presents a highly specialised pollination system: with asymmetric zygomorphic flowers, secondary pollen-presentation with the aid of a basket on the style apex, and stigmatic glue involved in pollen transference. A hinged carina on the lower petal protects the style and the pollen. The precision in both pollen economy and in reproductive isolation of two sympatric species.

I am grateful to Dr. GERHARD GOTTSBERGER and his family for their kind invitation, and to all other colleagues of the Department of Botany of the University of Botucatu for their friendly assistance during the research. The Department of Botany, Botucatu, São Paulo, offered research facilities. Prof. Dr. L. VAN DER PIJL was an invaluable stimulant during the research. S. A. CORBET, Cambridge, kindly corrected the English text.

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