

## Stigmatic cuticle in *hedysarum glomeratum*: structure and function

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**ABSTRACT** Plants of *Hedysarum glomeratum* F.G. Dietrich are self-compatible and fail to set seeds when protected from pollinators although its flowers are homogamous. Nevertheless, the stigma becomes receptive and self-pollination takes place if it is subjected to mechanical damage. In an ultrastructural study, we can appreciate that secretory products of the stigma are held beneath a cuticle even in very mature untouched stigmas. These products are not released to support pollen germination until the cuticle is broken. This mechanical barrier could prevent the spontaneous self-pollination whereas a pollinator does not visit the plant. Only when pollinators visit the flowers, both self and cross-pollination are possible. This mechanical system avoids to a large degree self-pollination in this annual herb.

### Introduction

Mature angiosperm pistils are differentiated into style and stigma. Stigma surface can be papillate or smooth having either a wet or dry surface. Dry and papillate stigmas can bear a superficial protein coating like the pellicle described in Caryophyllaceae or in Cruciferae (Heslop-Harrison & Heslop-Harrison, 1975). In these stigmas, pollen grains must penetrate this barrier to carry out the pollination. However, smooth and wet stigmas are covered by a variable amount of exudates. This exudate is composed by lipids, phenols, carbohydrates, amino acids and proteins (Baker *et al.*, 1974). Leguminosae have a smooth wet stigma and the secretion is retained by a cuticle until its mechanical rupture. This mechanical system has been described in *Trifolium pratense* (Heslop-Harrison & Heslop-Harrison, 1983) providing a protection against premature selfing. Nevertheless, this cuticle has not been described previously in the tribe Hedysareae. *Hedysarum* plants are homogamous, having the anthers and stigmas ripening at the same time, therefore spontaneous self-fertilization could be possible. *H. humile* and *H. coronarium*, both perennial herbs, are described to be self-incompatible (Prados, 1988) being the incompatible pollen tube totally arrested. Nevertheless, *H. spinosissimum* and *H. glomeratum*, both annual herbs, are self-compatible (Prados, 1988) producing no more than 4 seeds per fruit. In this report we describe the structure of the stigma in *H. glomeratum*. A seed set test in disturbed and undisturbed flowers is also carried out to demonstrate the role of the cuticle against premature self-fertilization.

### Material and Methods

Several tests were carried out to estimate the seed set under the following conditions: 1.- Three groups of plants were caged and protected from the wind. The first group (8 plants) was remained

untouched and the seed set was counted from 64 flowers. A total of 50 flowers from the second group (8 plants) were disturbed by touching the corolla with a small stick. We tried to simulate the behaviour of the pollinators with this disturbance. Obviously, only self-pollination is possible with this test. Seventy eight flowers from the third group (11 plants) were self-pollinated by depositing fresh pollen over the stigma. Fifteen plants were kept in wild open pollination and the seed set from 116 flowers were counted. Reproductive success was calculated by the following formulae:  $Sx100/Fx4$  being S the number of the seed set per flower and F the number of flowers considered. Stigmatic receptivity was tested over young to senescent stigmas following the unspecific esterase method (Pearse, 1972). Ultrastructure was examined by fixing styles at several stages of development in a mixture of 3,75% glutaraldehyde, 3% paraformaldehyde and 3mM calcium chloride in 0,1 M phosphate buffer (pH 7,4) for 2 hours. Sample material was post-fixed in a 1:1 mixture of 2% osmium tetroxide and 1.6% K<sub>3</sub>Fe(CN)<sub>6</sub> for 6 hours. After dehydration, the styles were embedded in Spurr's resin, cut in a Reichert Ultracut microtome, stained with uranyl acetate and lead citrate and examined with a Philips CM 10 transmission electron microscope.

### Results

The results of the different treatments to estimate the seed set are in Table I. No seeds were set in the 65 undisturbed flowers. When the flower is disturbed with the small stick, up to 11% of reproductive success is obtained. In most cases only one seed per fruit was set. Reproductive success in 78 hand self-pollinated flowers was 29,8%. A 20% more than in self-pollination was obtained in wild open pollination (50,3%).

Figure 1 shows different stages of the stigma of *H. glomeratum*. Figure 1a shows a detail of an epidermal cell from a young stigma (flower bud, 8mm). The cuticle layer is completely formed and

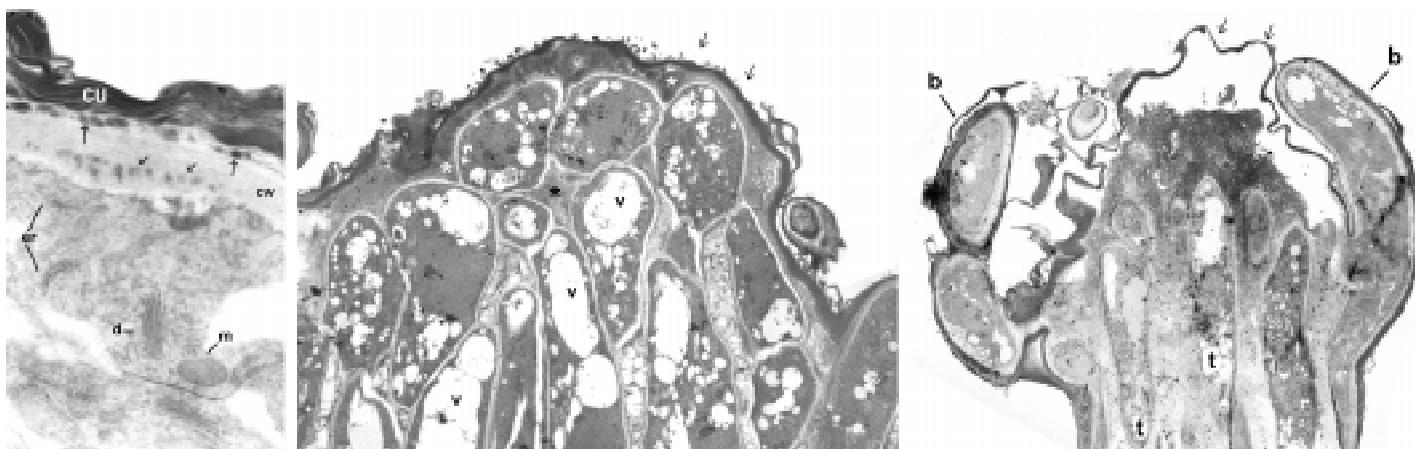
TABLE I

NUMBER OF FLOWERS, SEED SET AND REPRODUCTIVE SUCCESS IN THE FOUR GROUPS OF PLANTS

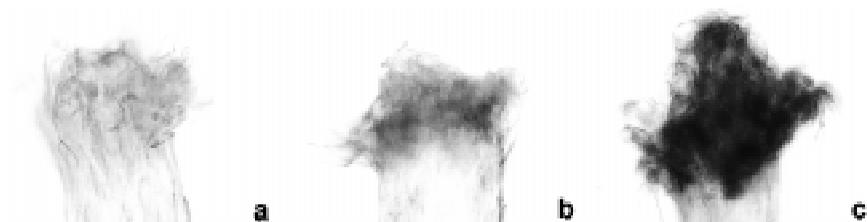
Type of treatment	Nº flowers	Nº seeds	Reproductive success (%)
Undisturbed flowers	65	0	0.0
Disturbed flowers	50	22	11.0
Self-pollination	78	93	29.8
Open-pollination	155	312	50.3

Reproductive success was calculated by means of the following formulae:  $Sx100/Fx4$  being S the number of seed set per flower and F the number of flowers considered.

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**Fig. 1. Different stages of the stigma of *H. glomeratum*.** (a) Detail of an epidermal cell from a young stigma (before anthesis). Osmiophilic granules (arrows) are deposited below the cuticle layer via exocytosis. cu, Cuticle; cw, cell wall; m, mitochondrion; er, endoplasmic reticulum; d, dyctiosome. X11.500. (b) Near to anthesis stigma. Exudate (asterisc) is being accumulated beneath the cuticle (arrows) and between intercellular spaces. Most transmitting cells are losing their cellular integrity. Large vacuoles (v) of possible holocrine excretion after the degeneration of protoplast. X1650. (c) Longitudinal section of a very mature stigma. The intact cuticle (arrows) is still present. A large amount of exudates are below the cuticle (asterisc). Most of the transmitting cells (t) are moribund. Brush hairs (h) adjacent to the receptive surface. X800.



**Fig. 2. Different stages of stigmatic maturation with the unspecific esterase method.** (a) Young stigma from an 8 mm flower bud. (b) Near to anthesis stigma. (c) Mature stigma from a flower in anthesis.

osmiophilic granules are produced in the plasmalema and deposited below the cuticle. These granules are secreted by the endoplasmic reticulum and excreted via exocytosis (Fig. 1a). Outside the cell wall they are accumulated forming the stigmatic exudates. A corresponding stage with the esterase test is in Fig. 2a. Only some parts of the stigmatic surface are slightly receptive. Fig. 1b shows a cross section of a young style (flower bud, 12mm near to anthesis). Transmitting cells are separated from each other because the secretion is accumulated within intercellular space. Most of the cells show large vacuoles, possibly of holocrine excretion, after degeneration of the transmitting tissue. An image of this stage with the test of esterase is in Fig. 2b. The reaction is specially intense in some parts of the stigmatic area where the secretion is accumulated. A longitudinal section of a mature stigma (from a flower after 3 days of anthesis) is shown in Fig. 1c. The stigmatic cuticle is still intact. Most transmitting tissue cells are lysated and the exudates fills all the structure. Esterase test shows that at anthesis all the head of the pistil is receptive (Fig. 2c).

## Conclusions

*Hedysarum glomeratum* has a wet and smooth stigma. Cuticle is formed at early stages of the stigma development by head stigma cells and always before the anthesis. Stigma exudates is synthesized by means of the endoplasmic reticulum and deposited via exocytosis beneath the cuticle and between the transmitting cells. The cuticle is still present even in very mature undisturbed pistils. The results

obtained in the different treatments indicates that self-pollination does not take place if the flower remains undisturbed. Only when the flower is disturbed the stigma becomes receptive to support pollen germination. A similar mechanical barrier seems to be in *H. glomeratum* to those described in *Trifolium pratense* (Heslop-Harrison & Heslop-Harrison, 1983). Premature self pollination is not possible if the cuticle is undamaged. As conclusion, mechanical rupture can be carried out by a pollinator during the anthesis and then both self- and cross-pollination are possible. Considering our results, the percentage of self-pollination after the visit of a pollinator is approximately a 11%, being the remaining possibilities to cross pollination. This mechanical system could be considered like a breeding system that increases the cross-pollination in an annual herb. Nevertheless, the reproductive success in open pollination (only 50%) indicates that other factors are affecting the seed production. Then further investigations are needed.

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