Phyton (Austria)	**Vol. 28**	Fasc. 2	193–200	15. 12. 1988

Stigma and Stigmatic Secretion Reexamined[1])

By

C. DUMAS*), R. B. BOWMAN**), T. GAUDE*), C. M. GUILLY*), Ph. HEIZMANN*), P. ROECKEL*) and M. ROUGIER*)

Received September 9, 1987

Key words: Stigma, stigmatic secretion, pollen-stigma recognition, stigma origin.

Summary

C. DUMAS, R. B. BOWMAN, T. GAUDE, C. M. GUILLY, Ph. HEIZMANN, P. ROECKEL & M. ROUGIER 1988. Stigma and stigmatic secretion reexamined. – Phyton (Austria) 28 (2): 193–200. – English with German summary.

An integral aspect of Angiosperm reproductive biology involves the spatial and temporal separation between pollen reception on the stigma surface and fertilization within the ovary. Various interactions between the male and female components serve to regulate the impending gametic fusion.

Thus the stigma, a unique glandular tissue, and its secretion play an active role during the programic phase of the fertilization. And, it is interesting to reexamine our knowledges on this special glandular tissue at the light of new data and concepts carried out by both evolutionary botany or molecular biology.

Zusammenfassung

C. DUMAS, R. B. BOWMAN, T. GAUDE, C. M. GUILLY, Ph. HEIZMANN, P. ROECKEL & M. ROUGIER 1988. Neuuntersuchung der Stigmen und der Stigma-Sekretion .–Phyton (Austria) 28 (2): 193–200. – Englisch mit deutscher Zusammenfassung.

Einen wesentlichen Aspekt der Reproduktionsbiologie der Angiospermen stellt die räumliche und zeitliche Trennung von Pollenrezeption an der Stigmenoberfläche und Befruchtung innerhalb des Eiapparates dar. Verschiedene Wechselbeziehungen

---

[1]) Invited lecture held on the XIV. International Botanical Congress 1987.

*) C. DUMAS, T. GAUDE, C. M. GUILLY, Ph. HEIZMANN, P. ROECKEL, M. ROUGIER, Université C. Bernard-Lyon I. UM CNRS 24, LA INRA, 43 Bd. du 11 Nov. 1918, F-69622 Villeurbanne CEDEX, France.

**) R. B. BOWMAN, Botany Department Colorado State University, Fort Collins, Colorado 80523, U.S.A.

zwischen den männlichen und weiblichen Komponenten dienen der Regulation der bevorstehenden Verschmelzung der Gameten.

So ist das Stigma ein einzigartiges Drüsengewebe und seine Sekretion spielt eine aktive Rolle während der progamischen Phase der Befruchtung. Es ist von Interesse, unsere Kenntnisse über dieses spezielle Gewebe im Lichte neuer, von der entwicklungsgeschichtlichen Botanik wie der Molekularbiologie beigebrachter Daten und Konzepte zu überprüfen.

Indroduction

The term of stigma has been quite clearly defined by PAYER in 1857, in his "Traité d'organogénie comparée de la fleur". This author claimed: "For me, I consider as stigma only the stylar region covered with papillate hairs". In this definition, there is obviously no indication related to the pollen reception and recognition by the pistil tissues. But, this primitive definition is very simple and thus clear. Later, CAPUS 1878, underlined the complex organization of the stigma and distinguished two different parts with the so-called "true stigma" more or less connected with the vascular trand of the style and the "collecting apparatus" constitued by the adjacent tissues. This distinction based on the role of the collecting apparatus in the pollination mechanisms and those of the "true stigma" in the pollen germination phenomena was quite approximative. But, the idea concerning a complex role played by the stigma tissue following its localization was suggested. And, today at the light of previous concepts provided as well by evolutionary botanists (BAILEY & SWAMY 1951, CORNER 1958) as by molecular biologists (CORNISH & al. 1987) it is interesting to reexamine the living stigma and the stigmatic secretion in Angiosperms. In addition, some ideas about artificial stigmas will be included in this paper.

1. Stigma types

1.1 Living stigmas

The first mention about the glandular activity of the stigma has been reported by GAERTNER 1844, in several genera as *Nicotiana, Datura, Physalis, Ribes, Malva* and *Lobelia* in which this author indicated the viscous or sticky nature of the exudate. Later BURCK 1901 classified the stigma types according to the presence or absence of liquid exudate at their surfaces. This distinction between wet and dry stigmas has been successively reused by several authors like KONAR & LINSKENS 1966, DUMAS 1973, VASIL 1974 and extensively reviewed by HESLOP-HARRISON 1981.

Stigma is a gland and its secretion is geared to the temporal processes of flowering and pollination. These processes have been well documented for the pistil of *Forsythia* by DUMAS 1973 with:

–A pre-secretory period following the pistil elongation (young flower buds)

–A synthetic period with an accumulation of endoplasmic reticulum profiles and vacuole enlargement in the stigmatic papillae

–An extrusion period more or less connected with the stigma receptivity.

The nature of liquid exudates in wet stigma types appears to vary widely between the different species with two main secretory mechanisms:

–A holocrine secretion observed in stigmas with a lipophilic exudate (DUMAS 1974, for example)

–A granulocrine seretion noticed in hydrophilic exudates (see KRISTEN & al. 1979, for example). But it seems that lipophilic secretion remains still obsure while granulocrine secretion types are much better understood (see criticism analysis in KNOX 1984).

In dry stigma types, i.e. *Brassica*, the pellicle is defined as the outermost extracellular coating of the stigma cells, and is a hydrophilic layer (MATTSON & al. 1974) resembling (but not analogous to) a biological membrane (GAUDE & DUMAS 1986).

Finally, the stigma provides the "read-out" system for incoming pollen "information", and thus possesses the receptor sites for pollen recognition (see DUMAS & al. 1984). And, several cytochemical activities have been identified in relationships with the pollen-stigma interactions, especially pollen adhesion and pollen hydration steps. Among these activities we may noticed several enzyme activities (esterase, ATPase), lectin-binding sites (ConA, Yariv antigen), non specific markers (lanthanum salts, cationized ferritin) (see review KNOX 1984). It is interesting to mention two new data: the presence of an adenylate cyclase activity only in compatible situation (ROUGIER in preparation) and the existence of nucleases (ROECKEL in preparation).

Silk of maize forming the stigma releases nucleases within the first minutes of contact with a liquid medium, and thus induces a complete degradation of exogenous DNA in less than 5 minutes. By another way, the diffusion of nucleases from maize pollen has been demonstrated by MATOUSEK & TUPY 1984. Thus, in maize pollen transformation both nuclease types diffuse from pollen and stigma and these enzyme activities have to be inhibated in order to get tranforming plants.

For these last experiments the stigma surface has to be receptive. Stigma receptivity is an important property during which it can be effectively pollinated. There are four classical methods currently available for assessing stigma receptivity:

–determination of seed set after pollination at different times relative to flower opening

–the presence of exudate

–cytochemical positivity for several tests: ATPase, lectin binding, . . .

–morphometric analysis of fruit development (for review see KNOX & al. 1986).

1.2 Artificial stigma

A working knowledge of those factors responsible for normal pollen function on a receptive stigma provides opportunity to model abiotic systems similarly capable of regulating male gametophyte function. And, the first artificial stigma was performed by KONAR & LINSKENS 1966, it is possible to surgically remove the stigma and style from a flower (BOWMAN 1984) and still achieve fertilization, provided that pollen placed on the stump can be induced to germinate and otherwise perform in a normal manner.

Successful synthetic stigma pollinations require the duplication of pollen influencing factors normally provided by the female reproductive tract. Despite the existence of various tests to measure pollen quality (HESLOP-HARRISON & al. 1984, KNOX & al. 1986), the only significant measurement of pollen function is its ability to achieve gametic union. Once a minimal composition for the synthetic stigma can be determined, additional components can be added and their direct impact on pollen function, as assessed by seed setting ability, can be measured. In most instances, the stigma-style tract is short lived and withers after fertilization. Similarly, synthetic stigmas principally influence only pollen behavior; any resulting seeds are left *in situ* to follow normal developmental pathways. The capacity to artifically manipulate pollen within the context of a normal pollination-fertilization scheme will become increasing by important with the isolation of S-allele products (ANDERSON & al. 1986). Synthetic stigmas will provide a mean for direct qualitative and quantitative measurement of influence from stigmatic fluid components, carbon sources and mineral requirements affecting pollen performance. Similarly, the ability to isolate stigma and style factors from those acting deeper in the ovary may confirm views that pollen tubes must pass through localized regions, each of which can influence pollen performance.

There is an ever growing body of literature suggesting that pollen of xenogamous species is subject to intense competition (MULCAHY & MULCAHY 1983). Only the best performers within the pollen population encounter unfertilized ovules; further, female imposed barriers (S-allele products), or genetic maladjustment (HOGENBOOM 1984) may prevent sucessful function of some or all of the pollen population arriving at the stigma. Inclusion of exogenous agents such as herbicides or toxins (BOWMAN 1985) within synthetic stigmas may also reduce the effectiveness of some pollen genomes. In this manner, artifical selection merely parallels natural selective processes. The tolerant pollen genomes capable of functioning in the presence of the stressing agents will be incorporated directly into seeds. Artificial selection for herbicide resistance for diverse haploid cell lines including fern gametophytes and tobacco cells (BRIGHT & al. 1986; CHALEFF & RAY 1984) have indicated the feasibility of pollen selections. Application of synthetic

stigma methods and other pollen selection systems (KNOX & al. 1986) will likely play a role in future pollen biotechnology.

2. Nature and origin of the stigma

2.1 Concepts and data from evolutionary botany

In his theoretical discussion of the nature of the stigma TOMAS 1934 arranged selected carpels of Rosaceae and of primitive genus as *Drimys, Schisandra, Trochodendron and Magnolia* in series illustrating two possible modes of origin of an apical stigma. BAILEY & SWAMY 1951 noticed that glandular hairs are widely distributed over the inner or ventral surfaces, as well as the margins of primitive forms of conduplicate carpels. During the phylogenetic closure of conduplicate carpels, the development of a style, and the restriction of paired external stigmatic crests to apical parts, the extensive internal glandular surfaces are retained and become variously modified as transmitting tissue.

On the other hand, according to CORNER'S concepts (1958) many evolutionary changes in plants including the division of labour and neoteny, result from restricting the site of development of a hereditary property or by moving it to another part of the plant body.

In this view, the cell-cell recognition mechanisms under the S-gene control occuring during pollen-pistil interactions are expressed in terms of rejection events at two different levels:

–the stigma surface in the sporophytic system, i.e. *Brassica* (see HESLOP-HARRISON & al. 1975, for example).

–the upper part of the style in the gametophytic system i.e. *Nicotiana* (see CLARKE al. 1985).

2.2 Concepts and data from molecular biology

In *Brassica oleracea,* a species with a sporophytic incompatibility system, the rejection of the pollen grain is controlled by the alleles of the diploid parent (sporophyte). The self pollen is recognized and rejected on the stigmatic surface. The S-molecules appear to be localized on the surface, could be in the pellicle. NASRALLAH & al. 1985 identified several stigma glycoproteins which segregate with the corresponding S alleles. These S glycoproteins are found only at the stigmatic surface, but not in the style or in vegetative tissues. Their synthesis is subjected to S-allele dosage. A second gene, termed M gene could be responsible for the rejection reaction (HINATA & OKAZAKI 1986).

DNA-sequence coding for these S-glycoproteins have been cloned in *B. oleracea* (NASRALLAH & al. 1985; NASRALLAH & al. 1987) from cDNA librairies made from stigma mRNAs. In addition the associated sequences have been deduced and compared from three S-glycoproteins from S-

alleles. They show the alternation of two constant (80 and 81% of homology) and two variable (41% and 46% of homology) regions, suggesting that the genes coding for the S-proteins could evolve by recombination between sets of related sequences (NASRALLAH & al. 1987, TAKAYAMA & al. 1987).

In gametophytic plants, the rejection of an incompatible pollen is controlled by its own haploid genome (gametophyte) and the rejection site is localized in the style. The corresponding S-glycoproteins can be detected all along the style, but their concentration is the highest at the level of the stigma and in the upper part of the style (*in situ* hybridization) (ANDERSON & al. 1986: CORNISH & al. 1987). The real function of the S-products seems to be dual since they participate in both recognition and rejection of the self pollen as seen in *Nicotiana alata*. In the gametophytic system, the events of rejection appear to be uncoupled from those of recognition. By contrast, these events occur simultaneous in the sporophytic plant species.

Conclusion

The stigma is a gland covered by specialized receptive cells able to recognize and to discriminate among pollen grains according to their genotype. And, the stigma in Angiosperms acts during interspecific matings to distinguish "not self", i.e., pollen belonging to a species other than that of the pistil is generally rejected, assuring maintenance of stability of the species. By contrast, in intraspecific matings, "not-self", which corresponds to allopollen, is accepted, while self-pollen is rejected. This latter process enforces outbreeding and characterizes "the self-incompatibility phenomenon" (GAUDE & DUMAS 1987).

On the other hand, *in situ* hybridization demonstrates an identical response in stigma cells, transmitting tissue and the inner epidermis of the ovary supporting previous ideas about stigma ontogenesis from evolutionary botanists.

Nevertheless, some key points remain to be solved: the precise localization of the S molecules on the stigma cells, their biosynthetic pathway and their molecular interactions with identical or complementary S molecules carried by the pollen grains.

Aknowledgements

We thank Anne-Marie Thierry for helpful technical assistance and INRA-CNRS contract Biotechnology MRES "Biologie moléculaire & cellulaire végétale" for financial support.

References

ANDERSON M. A., CORNISH E. C., MAU S. L., WILLIAMS E. G., HOGGART R., ATKINSON A., BONING I., GREGO B., SIMPSON R., ROCHE P. J. HALEY J. D. PENSCHOW J. D., NIALL H. D. TREGEAR G. W., COGHLAN J. P., GRAWFORD R. J. & CLARKE A. E. 1986. Cloning of cDNA for a stylar glycoprotein associated with expression of self-incompatibility in *Nicotiana alata*.–Nature 321: 38–44.

BAILEY I. W. & SWAMY B. G. L. 1951. The conduplicate carpel of dicotyledons and its initial trends of specializations.–Amer. J. Bot. 38: 373–379.

BOWMAN R. N. 1984. Experimental non-stigmatic pollinations in *Clarkia unguiculata* LINDL. (*Onagraceae*).–*Amer J. Bot. 71: 1338–1346.*

BOWMAN R. N. 1985. Factors influencing artificial gametophyte selections using synthetic stigmas.–In: MULCAHY D. L., MULCAHY G. B. & OTTAVIANO E. (Eds.), Biotechnology and Ecology of Pollen, p. 113–118.–Springer Verlag, New York.

BRIGHT J. W., OOMS G., FOULGER D., KARP A. & EVANS N. 1986. Mutation and tissue culture. In: WITHERS L. A. & ANDERSON P. G. (Eds.), Plant Tissue Culture and its Agriculture Applications, p. 431–449.–Butterworths, London.

BURCK W. 1901. Preservaties on the stigma against the germination of foreign pollen.–Proc. Koninkl. Ned. Akad. Wetenschap. 3: 264–274.

CAPUS G. 1878. Anatomie du tissu conducteur.–Ann. Sci. Nat., Bot., ser. VI, 7: 209–291.

CHALEFF R. S. & RAY T. B. 1984. Herbicide resistant cultures from tabacco cell cultures.–Science 223: 1148–1151.

CLARKE A. E., ANDERSON M. A., BACIC T., HARRIS P. J. & MAU S. L. 1985. Molecular basis of cell recognition during fertilization in higher plants.–J. Cell Sci. Suppl. 2: 261–285.

CORNER E. J. H. 1958. Transference of function.–J. L. S. B. 56: 33–40.

CORNISH E. C., PETTITT J. M., BONIG I. & CLARKE A. E. 1987. Developmentally controlled expression of a gene associated with self-incompatibility in *Nicotiana alata.*–Nature 326: 93–103.

DUMAS C. 1973. Contribution à l'étude cytophysiologique du stigmate: I-les étapes observées durant les processus glandulaires chez une *Oleaceae: Forsythia intermedia* Z.–Z. Pflanzenphysiol. 69: 35–54.

DUMAS C. 1974. Some aspects of stigma secretion in *Forsythia.*–In: LINSKENS H. F. (Ed.). Fertilization in higher Plants, p. 119–126.–Amsterdam.

DUMAS C., KNOX R. B. & GAUDE T. 1984. Pollen-pistil recognition: New concepts from electron microscopy and cytochemistry. In: BOURNE G. H. & DANIELLI J. F. (Eds.). Internat. Rev. Cytol., suppl. Pollen: Cytology and development, Vol. 90, p. 239–272.–Academic Press.

GAERTNER C. F. 1844. Beiträge zur Kenntnis der Befruchtung der Vollkommenen Gewächse. Versuche und Beobachtungen über die natürliche und künstliche Befruchtung durch den eigenen Pollen, p. 1–644.–E. Schweizerbarth, Stuttgart.

GAUDE T. & DUMAS C. 1986. Organization of stigma surface components in *Brassica* Cytochemical study.–J. Cell Sci. 82: 203–216.

GAUDE T. & DUMAS C. 1987. Molecular and cellular events of self-incompatibility.–In: GILES K. & PRAKASH J. (Eds.), Pollen: Cytology and Developement. Intern. Rev. Cytol., vol. 107, p. 333–366.–Academic Press.

HESLOP-HARRISON J., HESLOP-HARRISON Y. & BARBER J. 1975. The stigma surface in incompatibility responses.–Proc. R. Soc. Lond. B. 188: 287–297.

HESLOP-HARRISON J., HESLOP-HARRISON Y. & SHIVANNA K.R. 1984. The evaluation of pollen quality and a further appraisal of the fluorochromatic (FCR) test procedure.–Theoret. Appl. Genet. 67: 367–375.

HESLOP-HARRISON Y. 1981. Stigma charakteristics and Angiosperm taxonomy.–Nord. J. Bot. 1: 401–420.

HINATA K. & OKASAKI K. 1986. In: MULCAHY, G. D. L., MULCAHY G. B. & OTTAVIANO E. (Eds). Biotechnology and Ecology of Pollen.–Springer Verlag, New York, pp. 185–190.

HOGENBOOM N. G. 1984, Incongruity: Non-functioning of intercellular and intracellular partner relationships through non-matching information.–In: LINSKENS H. F. & HESLOP-HARRISON J. (Eds.), Encyclop. of Plant Physiology. New Series, Vol. 17, Cellular Interactions, p. 650–654.–Springer Verlag, Berlin.

KNOX R. B. 1984. Pollen-pistil Interactions.–In: LINSKENS H. F. & HESLOP-HARRISON J. (Eds.), Encyclopedia of Plant Physiology New Series, Vol. 17: Cellular interaction, p. 508–608.–Springer Verlag, Berlin.

KNOX R. B., WILLIAMS E. G. & DUMAS C. 1986. Pollen, pistil and reproductive function in crop plants.–In: JANICK J. (Ed.), Plant Breeding Rev. 4: 8–79.

KONAR R. N. & LINSKENS H. F. 1966. Physiology and biochemistry of the stigma fluid of *Petunia hybrida*.–Planta 71: 372–387.

KRISTEN U. BIEDERMAN M., LIEBEZEIT G., DAWSON R. & BOHN L. 1979. The composition of stigmatic exudate and the ultrastructure of the stigma papillae in *Aptenia cordifolia*.–European J. Cell Biol. 19: 281–287.

MATOUSEK J. & TUPY J. 1984. The release and some properties of nucleases from various pollen species.–J. Plant Physiol. 119: 169–178.

MATTSON O., KNOX R. B., HESLOP-HARRISON J. & HESLOP-HARISON Y. 1974. Protein pellicle of stigmatic papillae as a probable recognition site in incompatibility reactions.–Nature 247: 298–300.

MULCAHY D. L. & MULCAHY G. B. 1983. Pollen selction: an overview.–In: MULCAHY D. L. & OTTAVIANO E. (Eds.), Pollen: Biology and Implications for Plant Breeding, p. 15–17.–Elsevier, New York.

NASRALLAH J. B., KAO T. H., GOLDBERG M. L. & NASRALLAH M. E. 1985. A cDNA clone encoding an S-locus specific glycoprotein from *Brassica oleracea* L.–Nature 318: 263–267.

NASRALLAH J. B. KAO H., CHEN C. H., GOLDBERG M. L. & NASRALLAH M. E. 1987. Amino acid sequence of glycoproteins encoded by three alleles of the S-locus of *Brassica oleracea*.–Nature 326: 617–619.

PAYER J. B. 1857. Traité d'organogénie comparée de le fleur.–Paris.

TAKAYAMA S., ISOGAI A., TSUKAMOTO C., UEDA Y., HINATA K., OKAZAKI K. & SUZUKI A. 1987. Sequences of S-glycoproteins products of the *Brassica campestris* self IC-locus.–Nature 326: 102–105.

THOMAS H. H. 1934. The nature and origin of the stigma: a contribution towards a new morphological interpretation of Angiosperm flower.–New Phytol. 33: 173–198.

VASIL I. K. 1974. The histology and physiology of pollen germination and pollen tube growth on the stigma and in the style.–In: LINSKENS H.F. (Ed.). Fertilization in higher Plants, p. 105–118.–American Elsevier, North-Holland.