The route of the pollen tube from stigma to ovule in Populus nigra : a new look

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The route of the pollen tube from stigma to ovule in *Populus nigra*: a new look

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Summary

The complete progress of the *Populus nigra* pollen tube from the stigma surface to the ovule micropyle of *P. nigra* has been examined recently by the classical ABF method and by a new scanning electron microscope connected to a cryogenic preparation system.

Key words : Pollen, stigma, ovule, Populus.

1. Introduction

Hybridization programmes in *Populus* are limited by incompatibility barriers whose cellular sites and molecular mechanisms have not yet been defined. In spite of studies dealing with incompatibility in *Populus* in the last decade (WILLING & PRYOR, 1976 ; STETTLER & AGER, 1984), reproduction events especially intra-pistillar phenomena have not been clarified yet. During an investigation of these events, we have examined the pollination pathway, especially the behaviour of the pollen tube from the stigma surface to the ovule. This work is part of a programme on interspecific incompatibility in *Populus*, involving both the INRA Station d'Amélioration, des Arbres forestiers (Orléans, France) and the Université de Lyon, Laboratoire de Reconnaissance cellulaire et d'Amélioration des Plantes (Villeurbanne, France) (GAGET *et al.*, 1984 b).

2. Materials and methods

Branches of *Populus nigra* bearing flower buds were forced and flowers were matured in growth chambers (at the Université de Lyon, Villeurbanne, France). Before pollination, pollen viability was checked using the FCR test (Fluorochromatic Reaction) (HESLOP-HARRISON & HESLOP-HARRISON, 1970).

The progress of the pollen tube was monitored on the stigmatic surface and in the ovary cavity by scanning electron microscope (SEM), JEOL 35 CF connected to an EMSCOPE SP 2000 cryogenic system (of the Centre de Microscopie Electronique appliqué à la Biologie et la Géologie, Université de Lyon, Villeurbanne, France). The entire flower was fixed by rapid freezing, fractured and gold coated at − 160 °C before observation. Pollen tubes were observed in the stylar tissue by the decolorized aniline blue fluorescence method (ABF) in cleared whole mounts of pistil (DUMAS & KNOX, 1983).

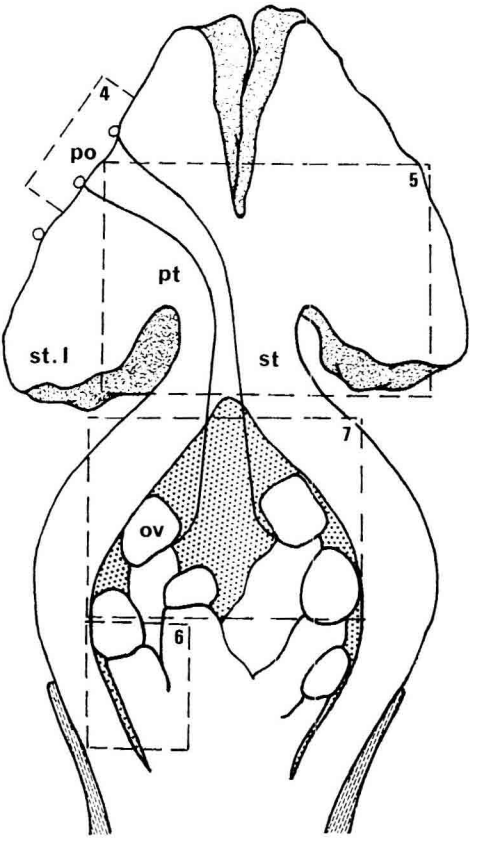

FIG. 1

Diagrammatic representation of a Populus nigra *flower.*
Représentation diagrammatique de la fleur de Populus nigra.

The areas 4 to 7 correspond to figures 4 to 7 of the plate. / *Les rectangles 4 à 7 correspondent aux figures 4 à 7 de la planche photographique.*

po : pollen grain / *grain de pollen.*
pt : pollen tube / *tube pollinique.*
st.l : stigmatic lobes / *lobes stigmatiques.*
st : stylodium / *stylode.*
ov : ovule / *ovule.*

3. Results

A *Populus nigra* catkin is composed of 30 to 40 flowers (fig. 2). A *Populus nigra* flower is represented in figure 1 and 3 : the bulbous ovary contains many ovules and is surmounted by four stigmatic lobes. Pollen grain adhesion, hydration and germination on a *P. nigra* stigmatic surface is observed in figure 4. Pollen tubes penetrate the stigma, grow through the stigmatic lobes and join together within the funnel-shaped stylar neck (stylodium, fig. 5). Somes tubes enter the ovary cavity, and grow on the inner surface (fig. 6). They reach the ovules by the funiculus (fig. 6), or directly if the ovule makes contact with the inner ovary wall. After 25 hours (20 °C) the pollen tubes pass through the micropyle of the ovules (fig. 7).

4. Discussion

Sexual reproduction in Poplars has been mainly focused on the observation of pollinated stigma surfaces (HAMILTON, 1976, STETTLER *et al.*, 1980). The behaviour of pollen tubes in the pistillar tissue has rarely been described (GAGET *et al,* 1984 a). In this paper we report the complete progress of the pollen tube from the stigma surface to the ovule micropyle for the first time for the genus Populus. The route of the pollen tube in the stigmatic tissue has been established by the classical ABF method. Complementary and original observations have been achieved in the ovary cavity by a scanning electron microscope, recently connected to a cryogenic preparation system. After rapid freezing, fresh pistil samples are fractured at low temperature before observation.

The visualization of the pollen tube progress in this cavity has raised the general problem of the guidance of the pollen tube to the micropyle of the ovule (see review by HESLOP-HARRISON & HESLOP-HARRISON, 1985). A directional change in the pollen tube of *P. nigra* at the base of the funiculus strongly suggests that part of the ovule may be chemotropically active towards the pollen tube.

Moreover, these two physiological methods have enabled us to establish pollen tube growth rate in *P. nigra* pistils (VILLAR *et al.*, 1986), and to reveal the sites of pollen tube arrest in an incompatible cross (GAGET *et al.*, 1984 a ; VILLAR, 1987). These modern investigations contribute to set the basic features of reproduction, and of male/female interactions in *Populus*.


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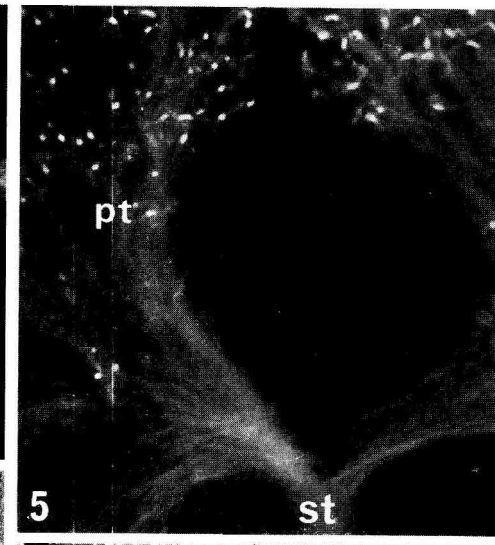
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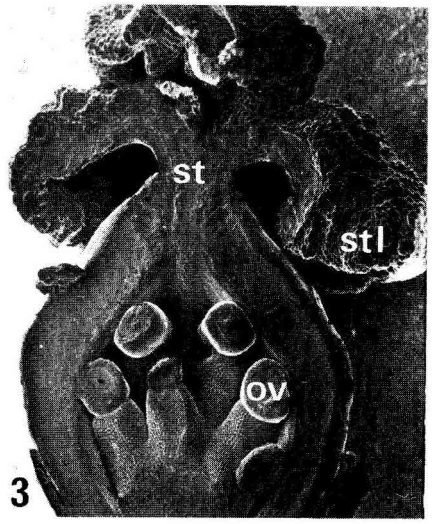
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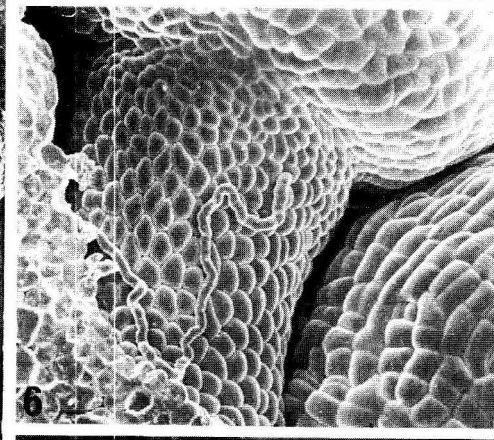
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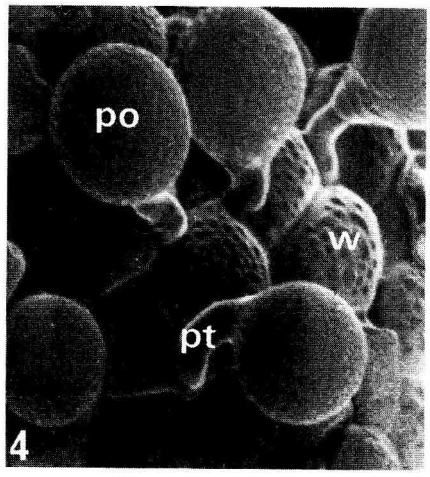
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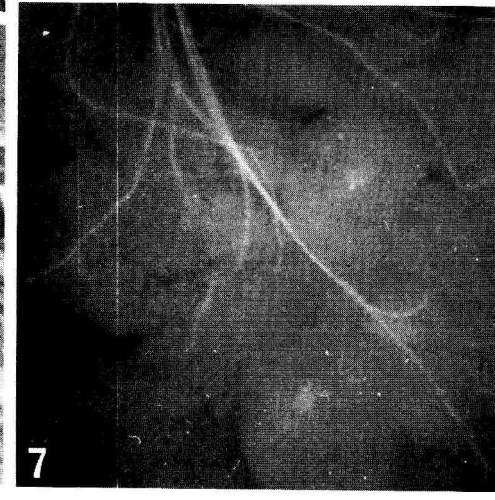
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FIG. 2

Female catkin of Populus nigra × *1,5.*
Chaton florifère femelle de Populus nigra × *1,5.*

FIG. 3

Longitudinal section of a female flower of Populus nigra *(SEM)* × *50.*
Section longitudinale d'une fleur femelle de Populus nigra *(Microcope électronique à balayage).*

st.l : stigmatic lobes / *lobes stigmatiques.*
st : stylodium / *stylode.*
ov : ovule / *ovule.*

FIG. 4

Detail of the pollinated stigmatic surface of Populus nigra *(SEM).*
Détail de la surface stigmatique pollinisée de Populus nigra *(MEB).*

Pollen grains germinated and pollen tubes have penetrated the stigma surface / *Les grains de pollen ont germé et les tubes polliniques ont pénétré la surface stigmatique.*

po : pollen / *grain de pollen.*
pt : pollen tube / *tube pollinique.*
w : waxes / *cires.*

FIG. 5

Fluorescent micrograph of stigmatic lobes of Populus nigra *(ABF method).*
Faisceaux de tubes polliniques dans les lobes stigmatiques se rejoignant au niveau du stylode (méthode ABF) × *150.*

Pollen grains have germinated (top of picture) and tubes can be seen traversing the stigmatic tissue towards the stylodium (base of picture) × 150.

pt : pollen tube / *tube pollinique.*
st : stylodium / *stylode.*

FIG. 6

Pollen tube growing on the funiculus of the ovule.
Tube pollinique en croissance le long du funicule de l'ovule.

Note the change in direction towards the ovule (SEM + EMSCOPE) × 440 / *A la base du funicule, le tube pollinique présente une brusque modification d'orientation en direction de l'ovule (MEB)* × *440.*

FIG. 7

Fluorescent micrograph of the ovary cavity of Populus nigra *(ABF method).*
Tubes polliniques dans la cavité ovarienne.

Pollen tubes tips have reached the ovules (25 h after pollination) × 70 / *Leur extrémité se localise au niveau des ovules (méthode ABF, 25 h après pollinisation)* × *70.*

Résumé

Cheminement du tube pollinique de P. nigra *du stigmate à l'ovule*

Nous avons examiné les principales étapes de la reproduction sexuée chez le Peuplier noir *(Populus nigra)*. Cette étude présente le cheminement du tube pollinique de *P. nigra* depuis la surface stigmatique jusqu'au micropyle ovulaire (fig. 1 et fig. 4 à 7). Sa progression a pu être observée par la technique classique de visualisation des tubes polliniques par le Bleu d'Aniline (méthode ABF) (fig. 5 et 7). L'utilisation du microscope électronique à balayage couplé à un système cryogénique EMSCOPE SP 2000 a permis des observations complémentaires originales sur le devenir du tube pollinique après pénétration dans la cavité ovarienne (fig. 6).

Mots clés : Pollen, stigmate, ovule, Populus.

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