

Pollination of the Genus *Hydrilla* (Hydrocharitaceae) by Waterborne Pollen Grains

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田中法生 : クロモ属 (トチカガミ科) における水面上花粉による送粉

Hydrilla (Hydrocharitaceae) is a monotypic genus containing only *Hydrilla verticillata* (L. f.) Royle. It is submerged, monoecious or occasionally dioecious, and distributed in the Old World and Australia; it has recently been naturalized in the New World. Its pollination mechanism has been reported to rely on the airborne transfer of pollen grains. The male flowers are liberated from the mother plant as buds, which then open explosively on the water surface, shooting pollen grains into the air. The hypanthium of female flowers elongates to the water surface, and the perianth opens and forms a funnel, which is hydrophobic. Pollen grains shot from male flowers were reported to be carried to the female funnel through the air, allowing pollination to take place (Ernst-Schwarzenbach 1945, Sculthorpe 1967, Cook 1982, 1988, Cook and Luond 1982, and Cook (1982) stated that pollen grains that land on the water surface are lost for reproductive purposes. Sculthorpe (1967) wrote that the possibility of water carrying pollen into the female flower is obviously precluded, because the peduncle of the female flower forms a funnel that is quite dry inside, with the three styles being surrounded by air, not water.

My field observations, however, revealed that pollen grains are transferred to female flowers via the water surface. I therefore presumed that pollen grains on the water surface also play a role in reproduction, as well as airborne pollen grains.

To verify this supposition, I first observed the pollination of *H. verticillata* in detail, and clarified whether pollen grains on the water surface were actually transferred to the stigmas of female flowers. Second, I used a 2, 3, 5-tetrazolium chloride test for enzyme activity to investigate the viability of pollen grains that dropped onto, and were transported by, the water surface. Third, I tested germination on the stigma to confirm that the pollen grains retained male fertility.

Materials and Methods

Plant material

Several male and female *Hydrilla verticillata* (L. f.) Royle plants were used for this study. They were collected from Shishizuka pond in Tsuchiura, Ibaraki Prefecture, Japan, and cultivated in Tsukuba Botanical Garden. Pollination was observed in an open-air aquarium.

Pollen viability test

A pollen viability test was carried out using pollen grains that had dropped onto the water surface and grains that had not. Mature male flower buds were collected onto the water surface in bottles. Pollen grains were used for enzyme tests at 0, 10 minutes, and 1, 2, 3, 4, 5, 10, or 20 hours after being

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dispersed on the water surface.

Enzyme tests depend on the presence of oxidation and on reduction reactions that are correlated with cell respiration. The procedures used followed the method of Cook and Stanley (1960). A drop of a 0.5% solution of 2, 3, 5-triphenyl tetrazolium chloride in 12% sucrose was placed on a microscope slide and pollen grains were added. A cover slip was then immediately applied to exclude oxygen. The slide was incubated at 55°C for 90 minutes before examination. Pollen grains stained red in the presence of reductases, indicating the presence of active enzymes. Red grains, which were therefore considered viable, were counted and staining rate was calculated.

Germination test on the stigma

Female flowers were collected four hours after hand pollination, which was done by dropping pollen grains onto the water surface around female flowers; care was taken that no pollen grains were transferred through the air. Each flower was placed on a microscope slide, stained with a drop of 0.1 % aniline blue in 0.1 M K_3PO_4 for at least 1 hour, then squashed with a cover slip and observed with an epifluorescence microscope.

Results

Observation of pollination

When a male flower bud has matured, the spathe tears and the bud is released to float on the water surface. The perianth soon opens (Fig. 1A), then each anther dehisces, scattering the pollen grains. Most of them drop onto the water surface within a radius of about 5 cm, but, if a female flower is nearby, some pollen grains are directly transferred to stigmas through the air, as Cook (1982) described.

The female hypanthium elongates to the water surface and the female flower opens, forming a funnel with the perianth (Fig. 1B). After landing on the water surface, pollen grains float and are then moved around by the wind or the flow of water. When they pass close to a female flower, they are quickly sucked into the funnel and transferred to the stigmas between two perianth segments (Fig. 1. C - F). This process transfers the majority of pollen grains, and pollination through the air is infrequent. The deep, vertical funnel that fresh flower usually forms is more suitable for catching pollen grains than the horizontal alignment that is usually seen in older flower.

Pollen viability

The staining rate of pollen grains that had not dropped onto the water was 94.4%. The rate after being on the water surface was 93.5% (10 minutes), 91.5% (1 hour), 84.2% (5 hours), 63.4% (10 hours) and 43.8% (20 hours) (Table 1). After 20 hours, 55.6 % of the pollen grains had burst, and these grains were excluded from the calculation.

Germination on the stigma

Pollen tubes were fully germinated on the stigma and extended into the style (Fig. 2). Most pollen grains germinated (data is not shown).

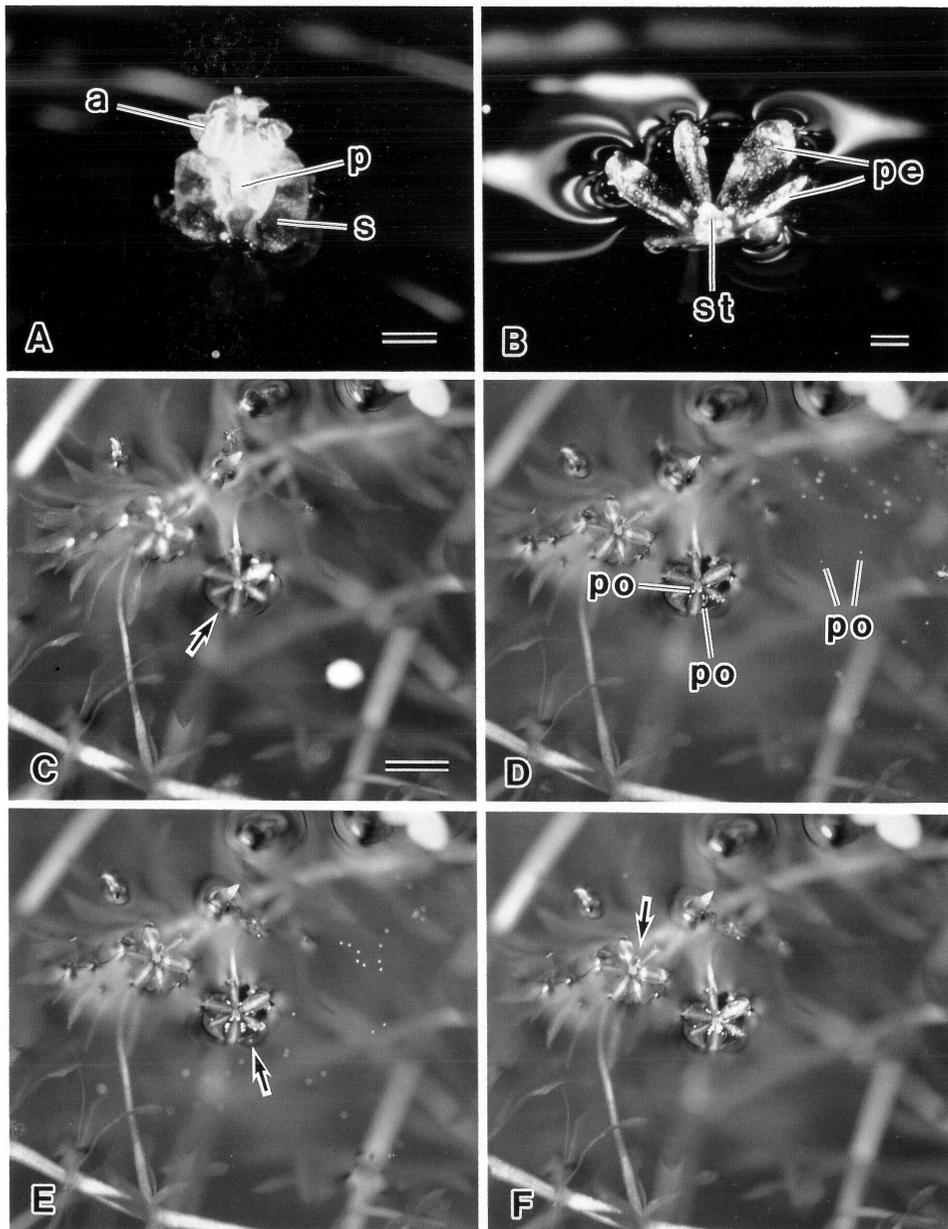


Fig. 1. Flowers and pollination of *Hydrilla verticillata*. **A.** Male flower on the water surface. Pollen grains have already been dispersed. a = anther, p = petal, s = sepal. **B.** Female flower on the water surface. Hypanthium is removed for taking a photograph. pe = perianth, st = stigma. **C - F.** A series of transfer of pollen grains on the water surface. **C.** Deep and vertical funnel of female flower (arrow shows) has already been pollinated by a few pollen grains. **D.** Some pollen grains were transferred to female funnel in a little while. po = pollen grain. **E.** More pollen grains were transferred. A pollen grain (arrow shows) is just being sucked into the funnel. **F.** Pollen grains transferred are gathered around the stigma. Horizontal funnel that is usually seen in older flower (arrow shows) has almost never been pollinated (see text).

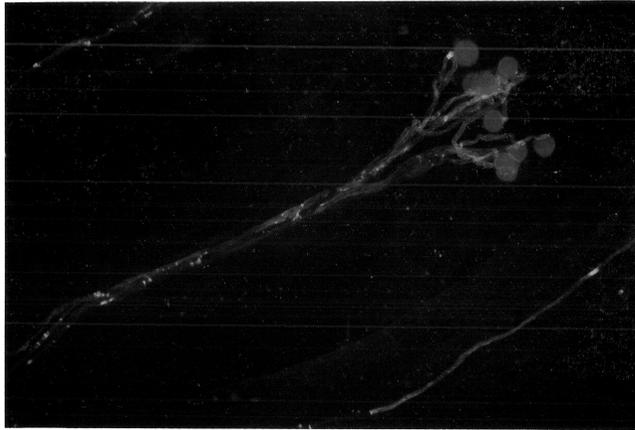


Fig. 2. Germination and extension of pollen grains into the stigma and the style four hours after pollination. Photograph by epifluorescence microscope.

Table 1. Staining rate of pollen grains of *Hydrilla verticillata*. Pollen grains were used for enzyme test at each time after being dispersed on the water surface.

	time								
	0 min.	10min.	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	10hrs.	20hrs.
staining rate (%)	94.4	93.5	91.5	97.5	90.2	76.1	84.2	63.4	43.8
(sample number)	(250)	(248)	(461)	(325)	(133)	(456)	(139)	(194)	(80)

Discussion

It was clear from the detailed observation of pollination that *Hydrilla verticillata* utilized the water surface rather than the air to transfer pollen grains. The test for pollen viability showed that about 80% of pollen stained up to 5 hours after dropping onto the water surface. Daumann (1963) examined the pollen viability of *Potamogeton* species and showed that the pollen of *P. natans*, a regular anemophilous species, rapidly loses its capacity to germinate on contact with water. On the other hand, the pollen of *P. lucens*, a submerged hydrophilous species, remained 45 % viable after a day in water. The pollen viability of *H. verticillata* is good compared with these species. In my observations, most grains reached a female flower within minutes, as pollen grains that dropped onto the water surface were almost always moved around by the wind or the flow of water. Although the time on the water will vary according to the environment and the proportion of male to female flowers in the population, 5 hours is probably usually sufficient to transfer the pollen grains to female flowers. Additionally, the pollen tubes germinated and extended into the stigmas very well, showing that pollen grains dropped onto the water surface also abundantly retain male fertility. I concluded that *H. verticillata* is mainly pollinated by waterborne pollen grains (epihydrophily).

I examined the floating time of pollen grains on the water surface, and found that 97.1% were

still floating even after 20 hours. Within them, 55.6 % of the pollen grains had burst. The factor inhibiting pollination is therefore likely to be the bursting or death of pollen grains, rather than their submergence.

Epihydrophily is uncommon in angiosperms. Some taxa have been reported as being plants that pollinate at or just above the water surface (epihydrophily): *Zostera marina* (Cox *et al.* 1992), *Potamogeton pectinatus* (Philbrick and Anderson 1987), *Halodule pinifolia*, *Halophila ovalis*, *Ruppia maritima* and *Lepilaena cylindrocarpa* (Cox and Knox 1989). The family Hydrocharitaceae has several epihydrophilous genera: *Vallisneria*, *Nechamandra*, *Lagarosiphon*, *Appertiella*, *Enhalus* and *Elodea* (Cook 1982, Cook and Triest 1982, Cook and Urmi-Konig 1985). In these taxa, however, only *Elodea* has a pollination mechanism similar to that of *Hydrilla*. The two genera have several characters in common: 1) The female flower elongates the hypanthium to reach the water surface. 2) The male flower is detached from the mother plant (or, in some species, attached to the mother plant by an elongated pedicel) and floats on the water surface. 3) The pollen grains are dispersed on the water surface. On the other hand, *Elodea* simply drops pollen grains on the water surface, whereas *Hydrilla* scatters pollen grains into the air. However, it is obvious that their pollination is very similar, and unusual in the Hydrocharitaceae or angiosperms. Although Cook (1982) divided the two genera into different categories on account of their pollination mechanisms, I suggest they should be treated as being in the same category.

Molecular phylogenetic analysis showed that monophyly of *Hydrilla* and *Elodea* was not supported (Tanaka *et al.* 1997, Les and Cleland 1997). Furthermore, Tanaka *et al.* (1997) made a character-state reconstruction of pollination mechanisms and concluded parallel evolution of epihydrophily. The specific mechanism that the two genera share is therefore regarded as having evolved in parallel.

Summary

It has been reported that pollination of *Hydrilla verticillata* (L. f.) Royle (Hydrocharitaceae) is carried out by pollen grains that have been shot into the air, and that pollen grains that fall on the water surface are not used for reproduction. However, field observations led me to believe that this was not the case and that pollen grains on the water surface are functional. I therefore observed pollination in detail, and investigated pollen viability and germination on the stigma. I found that pollen grains floating on the water surface were moved by the wind or the flow of water and then sucked into the funnels of female flowers. The majority of pollen grains were transferred by this process; airborne pollination was infrequent. During a test of pollen viability, about 80% of pollen grains were positive after up to 5 hours on the water surface. Pollen tubes were fully germinated on the stigma and extended into the style. The results show that *Hydrilla* is mainly pollinated by water-borne pollen grains (epihydrophily).

摘 要

これまでクロモ (トチカガミ科) は、葯から空中に飛散される花粉によって送粉するとされ、水面に落下したものは利用されないと記述されてきた。しかし筆者の野外観察から水面の花粉の

有効性が推測されたため、詳細な観察、水面花粉の生存及び発芽能力の調査を行い検証した。その結果、水面に落下した花粉は風や水の動きによって漂流した後、雌花の花被片の内側へ吸い込まれて柱頭に付着することがわかった。これは空中を移動するよりも頻繁に行われていた。また、水面へ落下した5時間後までは約8割の花粉生存率を示した。落下花粉の柱頭上での発芽及び伸張は良好であった。以上の結果、クロモ属は主に水面を媒体として送粉を行っていることが明らかになった。

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