Pollen Viability and Longevity in Two Species of *Arum*

Marc Gibernau* and David Macquart
Laboratoire d'Evolution & Diversité Biologique
Université Paul Sabatier
118 Route de Narbonne
Bât 4R3—B2
31062 Toulouse Cedex 4, France
*:gibernau@cict.fr

Anita Diaz
School of Conservation Sciences
Bournemouth University
Dorset House, Talbot Campus
Fern-Barrow, Poole
Dorset, BH12 5 BB, England

ABSTRACT

Pollen-loaded insects are not obligatorily captured rapidly by odoriferous inflorescences after their escape from a 'pollen-donor' inflorescence, but may be caught two or three days later. In such a situation, can these insects be considered as pollinators (i.e., pollen vectors) or just visitors?

Our results confirm that pollen grains in both species *Arum italicum* and *A. maculatum* quickly lose their viability. In natural conditions, pollen must then be dispersed quickly between male phase and female phase inflorescences in order for the pollination to be efficient. In fact, it should happen during the first hours after female *Psychoda* are liberated by male phase inflorescences. This is because pollinators captured on subsequent days would most probably carry non-viable pollen and thus would not pollinate the inflorescence they visit.

In natural conditions, pollen grains were viable for two days. By contrast, refrigerated pollen was viable for a longer time (4–5 days). Thus refrigeration at 8 or 15°C appears to be a good method to store pollen and prolong its viability.

KEY WORDS

Araceae, *Arum maculatum, Arum italicum,* pollination, pollen viability, pollen storage, *Psychoda.*

INTRODUCTION

Sapromyophily is defined as pollination systems involving insects (mainly flies) associated with decaying organic matter, which are based on deceiving pollinators by mimicking oviposition sites (Proctor *et al.,* 1996). This pollination system is known in species in the families Araceae, Aristolochiaceae, Asclepiadaceae and Orchidaceae (Proctor *et al.,* 1996). One of the first examples studied was *Arum nigrum* (Araceae), which attracts dung flies and carrion beetles (Knoll, 1926). As pollination is achieved by deception, trap mechanisms are needed in order to keep the attracted insects in the inflorescences (or flowers) during several hours or days. The inflorescences (or flowers) are protogynous, and the insects participate in both the female and male phases of the same inflorescences. Insects are attracted during the female phase (ovule fertilization) and released hours or days later during the male phase (pollen release). *Arum* species trap their pollinators thanks to the particular morphology and organisation of their inflorescences (Lack & Diaz, 1991; Boyce, 1993). Fertile male and female flowers are enclosed in a floral chamber at the base of the spathe. This chamber is closed in its upper part by horizontal hairs (often mod-

Table 1.   Composition of the Brewbaker & Kwack medium (1963) used for the germination tests of pollen grains of *Arum italicum.*

	MM (g/mol)	Conc. (mM)	Conc. (mg/l)
$Ca(NO_3)_2$ $4H_2O$	236.15	1.27	300
KNO_3	101.1	0.99	100
H_3BO_3	61.83	0.1	6.183
Saccharose	345	0.29	100
Agar 10 g/l pH 5.5			

ified sterile male flowers). Attracted insects fall into the floral chamber when only the female flowers are receptive, but cannot escape because of the hairs. The next day, after the emission of pollen, these hairs wither allowing the insects loaded with pollen to escape.

In western Europe, the pollination of two species, *Arum maculatum* and *A. italicum,* by psychodid flies has been studied (Prime, 1960; Lack & Diaz, 1991; Albre *et al.,* 2003). Various species in the genus *Psychoda* are involved. The flies are attracted during the female phase by a warm and odoriferous appendage, the distal part of the inflorescence, which mimics their brood site odour (dung or urine-like) (Bermadinger-Stabentheiner & Stabentheiner, 1995; Kite *et al.,* 1998). As pollination is achieved by deception, a trapped insect may not be willing to visit another inflorescence after its escape from the previous one. Thus pollen-loaded insects are not obligatorily captured on the same day of their escape by an odoriferous inflorescence, but may be caught two or three days later. In such a situation, can these insects be considered as pollinators (e.g. pollen vectors) or just visitors? One way to answer this question is to establish if pollen grains carried by pollinators are still viable after 2, 3 or more days. The aim of this paper is to test how long *Arum* pollen grains are viable.

There are direct and indirect measures of pollen viability. Direct tests consist of depositing the pollen on receptive stigmas and determining whether seeds are produced. Pollen germination can also be scored *in vitro.* Indirect methods rely on the correlation between ability to fertilize an ovule and some physiological or physical characteristics that can be determined more rapidly: (1) the fluorochromatic procedure (FCR), (2) testing pollen for enzyme activity, and (3) testing stainability of vegetative cells. The correlation is greatest for FCR and lowest for stainability (Heslop-Harrison *et al.,* 1984). The viability of pollen grains of two species (*Arum italicum* and *A. maculatum*) has been studied by direct tests using two experimental methods (*in vivo* and *in vitro*).

MATERIAL AND METHODS

In vitro—Arum italicum (Toulouse, France)

Pollen viability of *Arum italicum* has been tested by germinations of pollen grains *in vitro* in suspension on a Brewbaker-Kwack medium (Brewbaker & Kwack, 1963), the composition of which is indicated in Table 1. Pollen grains were soaked in a liquid medium or in distilled water above a solidified Brewbaker-Kwack medium on the bottom of the petri dishes.

Pollen from 18 inflorescences was collected in Eppendorf tubes between May 9th and May 22nd 2002. Two experiments were conducted on this pollen. Thirteen of the samples were stored in dry conditions at room temperature, and the remaining 5 were stored in a refrigerator (8°C). From each pollen sample, a first sub-sample was placed in the germination medium on the day of its release (t = 0) and other sub-
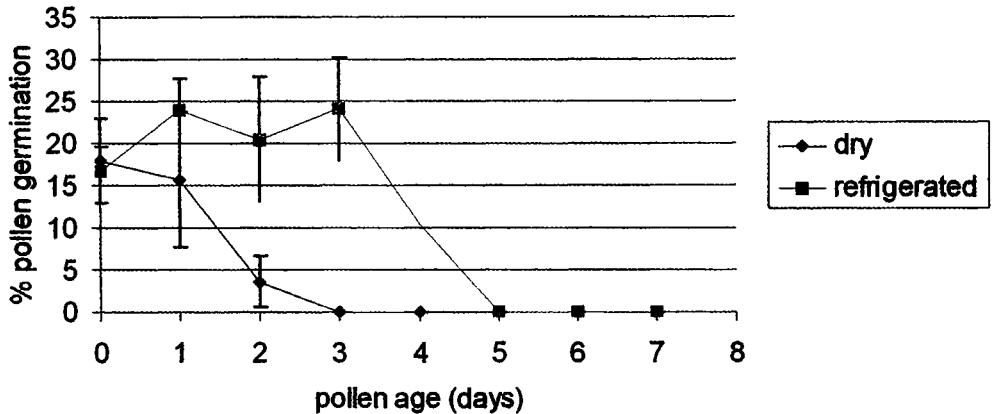
Arum italicum

Fig. 1.   Change of pollen germination rate at different days after pollen release, according to two modes of pollen conservation: dry condition (ambient temperature) or in a refrigerator (8°C).

samples 24 hours, 48 h, 72 h, 120 h, 144 h, and 168 h later.

In vivo—Arum maculatum (Dorset, England)

Pollen viability was tested by germinations of pollen grains *in vivo* by measuring the success of seed set of hand-pollinated plants where pollen varied in age. All pollen donors were from single genotype, outcrossed sources. Pollen was applied liberally to all stigmas in the inflorescence during early female phase.

Two experiments were conducted. The pollen was stored either in a dark, dry cupboard at 15°C or in the field inside the spathes under natural conditions. For both experiments ten inflorescences were hand-pollinated and the success of berry set was measured as a percentage of berries containing seed per inflorescence.

RESULTS

In vitro—Arum italicum

Germination rates were similar when pollen grains were soaked in a liquid Brewbaker-Kwack medium to when they were soaked in distilled water. Germination- rate patterns were very different be-

tween the two experiments as shown in Fig. 1. The main results are:

1) The pollen germination rate was low at t = 0: ranging from 15 to 32%.
2) Pollen stored in Eppendorf tubes in dry conditions and at room temperature has lost its viability in 48 hours.
3) Pollen stored in a refrigerator remained viable (20–25%) for three days (72 hours) then its viability decreased rapidly to zero.

In vivo—Arum maculatum

Germination rate patterns were very different between the two experiments as shown in Fig. 2. The main results are:

1) High pollen germination rates (100%) were obtained at the beginning of both experiments.
2) Pollen stored in the field within the spathe remained highly viable for two days, decreasing on the third day (36%). Pollen viability reached zero on the next and following days.
3) Pollen stored at 15°C remained highly viable for 4 days, decreasing on the fifth day to 51%. Pollen remained weakly viable (10%) on the two subsequent days.
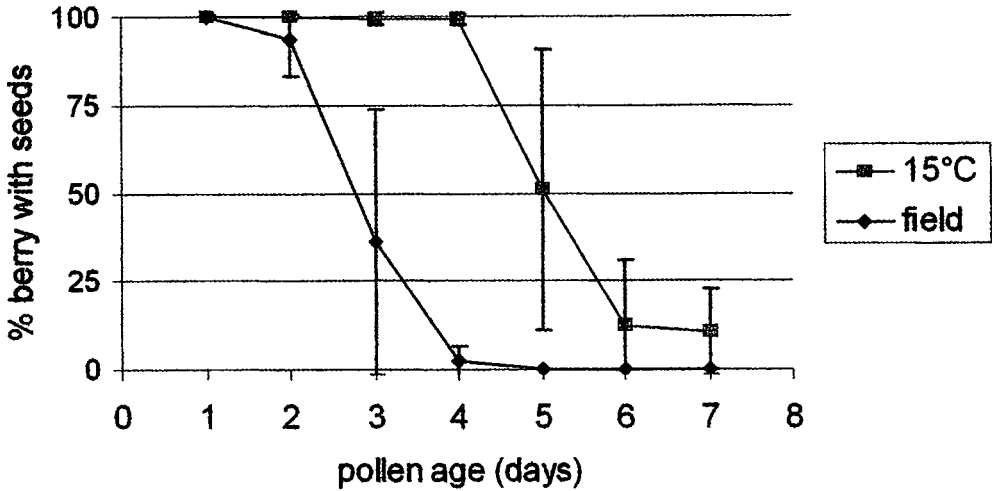
Arum maculatum

Fig. 2.   Change of pollen germination rate at different days after pollen release, according to two modes of pollen conservation: field condition within spathes (ambient temperature) or in a cupboard (15°C).

DISCUSSION

Germination of pollen is generally most successful immediately after anthesis, and viability deteriorates rapidly in most species (Kearns & Inouye, 1993). In *Erythronium grandiflorum* (Liliaceae), pollen viability decreases significantly within an hour of exposure to the air after dehiscence (Kearns & Inouye, 1993). However, not all pollen is short-lived; some Rosaceous and Liliaceous pollen can remain viable for 100 days (Leduc *et al.*, 1990). Our results confirm a "rapid" pollen viability loss for *Arum italicum* and *A. maculatum*. In natural conditions ("field", Fig. 2), pollen grains were viable for 2 (-3) days whereas in semi-natural conditions ("dry", Fig. 1) for only 1 (-2) day(s). By contrast, refrigerated pollen was viable for longer times (4–5 days).

In order to hand pollinate *Amorphophallus paeoniifolius,* pollen grains were stored under high humidity in a refrigerator, but viability is extended for only a short time by this method (Hyndman, 2001). The pollen viability of *Clarkia* (On-agraceae) decreases with time at room temperature both on the plant or in the lab, but pollen stored at 5°C retained its ability to fertilize ovules for longer periods (Smith-Huerta & Vasek, 1984). Thus refrigeration at 8 or 15°C appears to be a good method to store and prolong pollen viability (two days in the two studied *Arum* species).

Pollen can be shed in the binucleate or trinucleate stage. Binucleate pollen germinates fairly easily whereas trinucleate pollen (for example in Asteraceae or Poaceae) has a very short life and is difficult to germinate *in vitro* (Grayum, 1986; Kearns & Inouye, 1993). The pollen of *Amorphophallus paeoniifolius* is trinucleate and sensitive to desiccation whereas *A. konjac* has a binucleate 'resistant' pollen (Hyndman, 2001). In the same way, *Arum italicum* and *A. maculatum* have trinucleate 'sensitive' pollen grains, moreover they are starchless, a rare combination in aroids (Grayum, 1986).

Our results suggest that the Brewbaker-Kwack medium may not be suited for

Arum italicum pollen to germinate, or that *Arum italicum* pollen has naturally a low viability. However, this last hypothesis is somewhat contradicted by our results on *A. maculatum* although we can not exclude differences in pollen viability among species. Pollen viability of *A. italicum* was studied by an "individual grain method" whereas it was by an overall ("population") method for *A. maculatum*. Brewbaker-Kwack medium has been used successfully for many species with binucleate pollen, but other species, particularly those in the Brassicaceae and Compositae, with trinucleate pollen require a different medium. Only 16% germination of *Capsella bursa-pastoris* (Brassicaceae) pollen was obtained with Brewbaker-Kwack medium whereas 47% of the pollen germinated in a more appropriate medium (Leduc *et al.*, 1990).

In nature, water, sugar and amino acids are supplied by the style to nourish the growing pollen tube. For many species, boron and calcium are also required for pollen tube growth. Boron, which is provided by stigmas and styles, facilitates sugar uptake and has a role in pectin production in the pollen tube (Richards, 1986). Calcium, found on the surface of some pollen grains, is often required for germination and has been implicated in the successful germination of a large number of pollen grains. Calcium is also involved in pectin synthesis and control of osmotic conditions (Richards, 1986). At least 79 genera require a medium containing sucrose, boric acid, and calcium.

Pollen viability in *Arum* (e.g. *A. italicum* and *maculatum*) appears to be short in "natural" conditions. Consequently, it must be dispersed quickly between mature and receptive inflorescences in order for the pollination to be efficient. In fact, in most of the cases, it should happen during the first 12 hours between female *Psychoda* being liberated by male phase inflorescences and the opening of fresh female phase inflorescences. In *A. maculatum* the pollinators are released in the morning and captured by fresh inflorescences the following evening whereas in

A. italicum female *Psychoda* are liberated in the early evening and captured later in the same evening by fragrant inflorescences. If this capture occurs the following day, the visit will still be positive (i.e. ovule fertilisation) as pollen grains are still viable. By contrast, pollinators captured on subsequent days, would most probably carry non-viable pollen and thus would not pollinate the inflorescence they visit.

Further experiments are needed to investigate whether pollen viability deteriorates rapidly in other species of *Arum* and Araceae, and whether large between-species variation in pollen viability explains the low rates observed in *Arum italicum*.

ACKNOWLEDGMENTS

LITERATURE CITED

Albre, J., A. Quilichini & M. Gibernau. 2003. Pollination ecology of *Arum italicum* (Araceae). *Bot. J. Linn. Soc.* 141(2):205–214.

Bermadinger-Stabentheiner, E. & A. Stabentheiner. 1995. Dynamics of thermogenesis and structure of epidermal tissues in inflorescences of *Arum maculatum*. *New Phytol.* 131:41–50.

Boyce, P. 1993. The genus *Arum*. A Kew magazine monograph. HMSO, London.

Brewbaker, J. L. & B. H. Kwack. 1963. The essential role of calcium ion in pollen germination and pollen tube growth. *Am. J. Bot.* 50(9):747–858.

Grayum, M. H. 1986. Phylogenetic implications of pollen nuclear number in the Araceae. *Plant Syst. and Evol.* 151:145–161.

Heslop-Harrison, J., Y. Heslop-Harrison & K. R. Shivanna. 1984. The evaluation of pollen quality, and a further appraisal of the fluorochromatic (FCR) test procedure. *Theor. & Appl. Gen.* 45:115–122.

Hyndman, S. 2001. Hand-pollination of *Amorphophallus paeoniifolius*. Web page:www.aroid.org/pollination/hyndman/index.html.

Kearns, C. A. & D. W. Inouye. 1993. *Techniques for Pollination Biologists*. University Press of Colorado, Niwot.

Kite, G. C., W. L. A. Hetterscheid, M. J. Lewis, P. C. Boyce, J. Ollerton, E. Cocklin, A. Diaz & M. S. J. Simmonds. 1998. Inflorescence odours and pollinators of *Arum* and *Amorphophallus* (Araceae), pp. 295–315 *In* S. J. Owens & P. J. Rudall (eds.), *Reproductive Biology*. Royal Botanic Gardens, Kew.

Knoll, F. 1926. Die *Arum*-blütenstände und ihre Besucher. *Abhandlungen der zoologisch-botanischen Gesellschaft in Wien* 12:379–481.

Lack, A. J. & A. Diaz. 1991. The pollination of *Arum maculatum* L.—a historical review and new observations. *Watsonia* 18:333–342.

Leduc, N., Monnier, M. & G. C. Douglas. 1990. Germination of trinucleated pollen: formulation of a new medium for *Capsella bursa-pastoris*. *Sex. Plant Reprod.* 3:228–235.

Prime, C. T. 1960. *Lords and Ladies*. Collins Clear-Type, London.

Proctor, M., P. Yéo & A. Lack 1996. The natural history of pollination. *In* S. A. Corbet, S. M. Walters, R. West, D. Streeter & D. A. Ratcliffe (eds.), *The New Naturalist*. The Bath Press, London.

Richards, A. J. 1986. *Plant Breeding Systems*. George Allen & Unwin, London, England.

Smith-Huerta, N. L. & F. C. Vasek. 1984. Pollen longevity and stigma pre-emption in *Clarkia*. *Am. J. Bot.* 71:1183–1191.