The viability of pollen grains of a lily *(Lilium auratum)* and the eggs of the brine-shrimp *(Artemia salina)* soaked in organic solvents for 10 years

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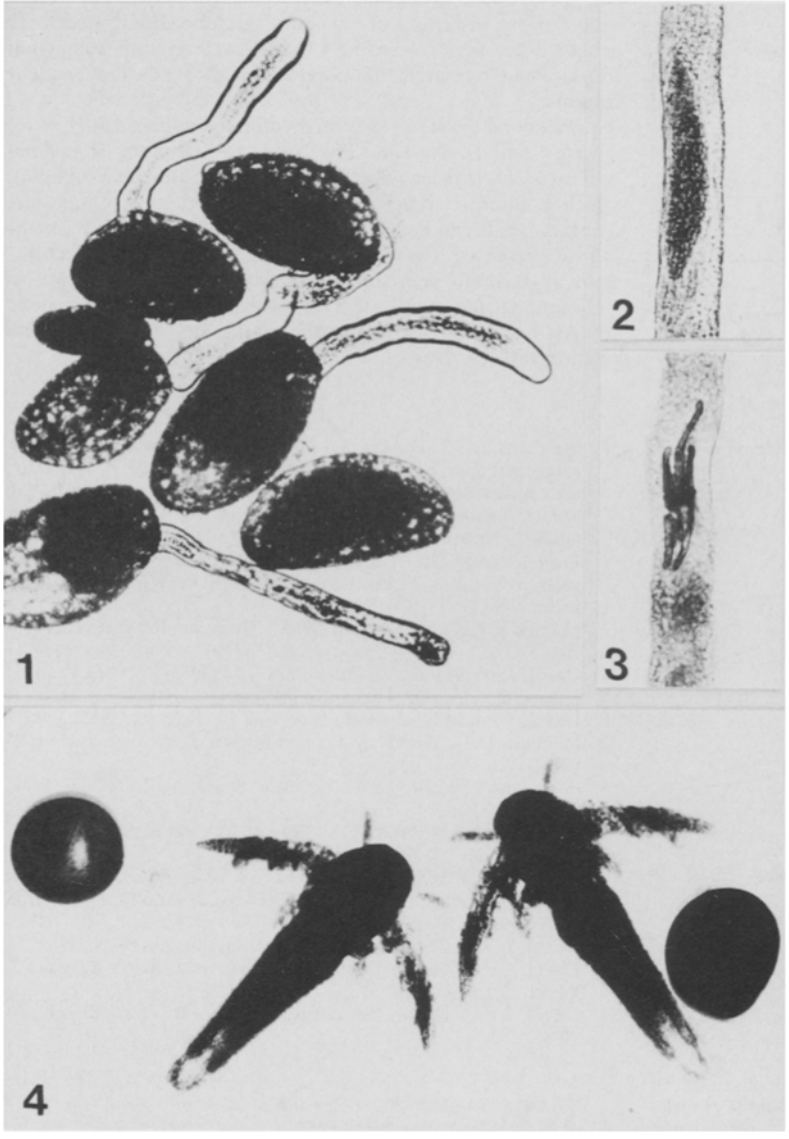
Summary. Pollen grains of *Lilium auratum* which had been stored in n-pentanol, n-butanol and n-propanol for 10 years at −10 °C germinated, and the generative nucleus in the pollen tube divided into 2 sperm nuclei. The resting eggs of *Artemia salina* soaked in these solvents for 10 years hatched at a high rate.

It is a matter of common knowledge in biology that organic solvents injure living cells or kill them. However, Iwanami observed that pollen grains of *Lilium, Camellia* and *Impatiens* soaked in organic solvents such as acetone, benzene, chloroform and ether for a short period germinated well[1–5]. It was further observed that the flowers of *Petunia hybrida* pollinated by *Petunia* pollen soaked in these organic solvents for 6 days produced many seeds[6] and that the seeds of various kinds of plants[7], resting eggs of brine-shrimp[8,9] and *Bacillus* spores [10] also retained their viability in these organic solvents.

The most noticeable result in the findings may be that the curve of the pollen longevity was separated into two phases by soaking in diethyl ether, namely, pollen grains soaked in diethyl ether became absolutely dormant temporarily, but recovered their initial activity when taken out of the solution[4]. This fact indicates that pollen can retain its viability in organic solvents for a longer period than expected. In this study, the viability of the pollen grains of a lily *(Lilium auratum)* and the eggs of the brine-shrimp *(Artemia salina)* soaked in organic solvents for 10 years was tested.

Lilium auratum pollen was collected from freshly opened flowers in Yokohama and stored in a plastic box with silica gel. After 24 h , 20 mg of pollen grains were soaked in 5 ml of organic solvent in a small test tube with a cork stopper and the tube was sealed with vinyl tape. The test tube was put in a freezer and kept at −10 °C. The resting eggs of *Artemia salina*



The photographs show that the pollen grains of *Lilium auratum* and the eggs of *Artemia salina* which had been soaked in n-pentanol for 10 years retained their viability. Figure 1. Germination of pollen grains (2 h after sowing on culture medium). Figure 2. Generative nucleus in the pollen tube (8 h after sowing). Figure 3. Chromosomes in the pollen tube (16 h after sowing). Figure 4. Hatching of eggs (after 5 days of soaking into sea water).

Viability of pollen grains of *Lilium auratum* and eggs of *Artemia salina* which had been soaked in various organic solvents for 10 years at 10 °C

Organic solvent	Germination of pollen grains (%)	Hatching of eggs (%)
Control (1) (fresh)	90.7	74.8
Control (2) (no soaking)	0	28.3
Methanol	0	0
Ethanol	0	0
n-Propanol	10.1	31.5
n-Butanol	55.2	62.2
n-Pentanol	49.5	69.7
Acetone	0	0
Diethyl ether	0	0
Xylene	0	0

of California origin were also soaked in organic solvent (20 mg/5 ml) and stored in the freezer. Organic solvents used in this study were methanol, ethanol, n-propanol, n-butanol, n-pentanol, diethyl ether and xylene (Tokyo Kasei Kogyo Co., Ltd). After 10 years, pollen grains or eggs in the solvents were filtered, and then desiccated using an aspirator for 10 min. About 200 pollen grains were taken from the filter paper and cultured on the medium (sucrose 10%; boric acid, 100 ppm; calcium nitrate, 300 ppm) at 25 °C. After 2 h, the germination percentage of the cultured pollen grains was measured. In the case of the eggs of brine-shrimp, about 100 eggs taken from the filter paper were cultured in 2.5 ml of artificial sea water in a small petri dish at 25 °C. After 5 days, the number of hatched shrimps was measured by the use of a small projector. The experiments were repeated three times; mean values are shown in the table.

As shown in the table, pollen grains of control (2) (no soaking) and those soaked in methanol, ethanol, diethyl ether and xylene for 10 years did not germiante at all, however, about 50% of pollen soaked in n-butanol and n-pentanol germinated (fig. 1). Pollen soaked in n-propanol germinated poorly (10.1%). In the case of the eggs of brine-shrimp, eggs of control (2) hatched poorly (28.3%) and eggs soaked in methanol, ethanol, acetone, diethyl ether and xylene did not hatch at all. However, the eggs of brine-shrimp soaked in n-butanol and n-pentanol hatched at a high rate (62.2 and 69.7%), and the eggs soaked in n-propanol hatched at a low rate (31.5%). The modes of hatching of the eggs of control (2) and those of soaked eggs in 3 organic solvents were almost same as control (1). In fact, it seems that these pollen grains and eggs are not injured by soaking in n-butanol or n-pentanol, because soaked pollen grains developed long pollen tubes, the generative nucleus translocated into the pollen tube divided to give 2 sperm nuclei (figs 2 and 3) and protoplasmic streaming of normal speed (2.6 μm/sec) was observed in the pollen tube. The brine-shrimps from eggs soaked in these solvents continued their normal motion for a long time in sea water (fig. 4). It has been observed that the eggs of brine-shrimp stored in organic solvents at low temperature (5 °C, −15 °C) retained their viability for a longer time than those stored at high temperatures (30 °C)[9]. Low temperature may be an important factor if the viability of pollens is to be retained in organic solvents for a long period. In this experiment, when the viability was tested after 1 year of soaking, the pollen grains and the eggs soaked in organic solvents other than methanol and ethanol germinated and hatched at a high rate. The reason why only the pollen grains and eggs soaked in n-butanol, n-pentanol and n-propanol retained their viability for more than 10 years is not known. However, the results of this study are consistent with the fact that when pollen grains of *Camellia japonica* and *Erythrina indica* were soaked in alcohols with 2 to 12 carbon atoms for 1 week at 5 °C, the best retention of viability was found with C_4 to C_6 alcohols[10]. Whether pollen grains stored in organic solvents for 10 years are still able to fertilize ovules or not is not known; however, lily flowers pollinated by lily pollen grains soaked in n-butanol, acetone, diethyl ether and xylene for 1 year at −10 °C produced many normal seeds. Though the mechanism by which these organisms retain their has not yet been clarified, further studies on the relation between retention of viability and the character of organic solvents may make it possible that the viability of other plant and animal cells can also be retained in organic solvents.

1 Iwanami, Y., Jap. J. Palynology *8* (1971) 39 (in Japanese).
2 Iwanami, Y., and Nakamura, N., Stain Technol. *47* (1972) 137.
3 Iwanami, Y., Pl. Physiol. *13* (1972) 1139.
4 Iwanami, Y., Protoplasma *84* (1975) 181.
5 Mishra, R., and Shivanna, K. R., Euphytica *31* (1982) 991.
6 Iwanami, Y., Botanique *4* (1973) 53.
7 Iwanami, Y., and Akizawa, K., Jap. J. Breed *24* (1974) 59 (in Japanese).
8 Iwanami, Y., Expl Cell Res. *78* (1973) 470.
9 Iwanami, Y., and Tazawa, E., Zool. Mag. *83* (1974) 267 (in Japanese).
10 Iwanami, Y., Inoue, J., Kojima, M., and Fukuda, T., Jap. J. Palynology *18* (1976) 19 (in Japanese).

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Correlation of aphid sex pheromone gland number with ovarian development

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Summary. The number of sex pheromone releasing glands located in female aphid hind tibiae has been counted in both oviparae with full normal vitellogenic ovaries and in ovipara/vivipara intermorphs bearing reduced numbers of vitellogenic eggs. The finding of a good correlation between vitellogenic eggs and pheromone gland number suggests that the ovary may control pheromone gland morphogenesis.

In holocyclic *Aphidina viviovipara* 2 reproductive female categories exist: the oviparous sexual and the viviparous parthenogenetic females. Oviparous and viviparous females differ mostly in their ovaries. In fact, even if the number of ovarioles is the same in both categories[1], the oviparous females maturate large, yolk-rich, haploid eggs, while the viviparous females bear very small diploid eggs which quickly develop by parthenogenesis.

In most species, during the sexual phase, males are attracted by the sex pheromone released through integumental glands located in the hind tibiae of the oviparous females. These organs appear as round plaques. Their function as sex phe-