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.

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Correlation of aphid sex pheromone gland number with ovarian development

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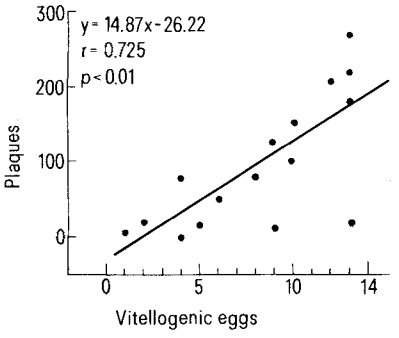
Summary. The number of sex pheromone releasing glands located in female aphid hind tibiae has been counted in both oviparae with full normal vitellogenic ovaries and in ovipara/vivipara intermorphs bearing reduced numbers of vitellogenic eggs. The finding of a good correlation between vitellogenic eggs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



Plot between the number of scent plaques and that of vitellogenic eggs in the viviparous/oviparous intermediate females.

among the first offspring of viviparae subjected to short photoperiods from 2 days before birth. The ovaries of the experimental females were examined in 9-day-old adults by dissection in saline. Scent plaque counts were performed on the same dissected insects, cleared in Andre's solution and mounted in a Berlese medium. Data were analyzed by means of the linear correlation significance test.

Results and discussion. Whenever an aphid bearing a mixed ovary was found the number of its vitellogenic eggs was recorded and the hind tibiae separated for plaque count. A total of 17 intermorph females were obtained during 4 experimental cultures in which the number of vitellogenic eggs ranged from 1 to 13. Scent plaque counts were performed, moreover, on viviparae from the basic culture and on oviparae with normal oviparous ovaries consistently bearing 14 vitellogenic eggs.

Table 1 shows the number of gland plaques in the 3 aphid morphs mentioned. It was confirmed that viviparae have no plaques at all. Ovipara/vivipara intermorphs differ from oviparae not only in the lower number of plaques, but also for the high standard deviation value of their mean. This high variability of intermorph plaque number can be correlated with the number of vitellogenic eggs in their mixed ovaries.

Table 1. Mean and standard deviation of pheromone plaque number per tibia in the 3 female morphs

Morph	Tibiae, n	Plaques, mean value	SD
Vivipara	40	0	0
Ovipara	40	337	25.5
Intermorph	34	91	87.5

Table 2. Number of vitellogenic eggs and scent plaques in 17 photoperiod-induced ovipara/vivipara intermorphs

Intermorph No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Vitellogenic eggs	5	6	10	8	2	1	10	4	2	13	12	9	13	4	13	13	9
Plaques	12	52	104	80	20	6	152	0	20	12	204	8	221	78	273	180	125

romone releasing pores[2-4] has been assessed by means of mating studies, the chemical nature of the sex pheromone being yet unknown. Owing to their function these organs are called scent plaques.

In oviparous females of *Acyrthosiphon pisum* (Harris) the scent plaques are very abundant and occupy the tibial integument almost completely. By the use of light microscopy they can easily be observed and counted. Viviparous parthenogenetic females, which obviously do not copulate, completely lack these organs.

Recently, it has been demonstrated that juvenile hormone (JH) plays an important role in regulating the morphogenesis of scent plaques in aphids[5-7]. It is not known, however, whether this effect is a direct action of the hormone on tibial plaques or an indirect one mediated by the endocrine system of the aphid. The experiments reported in this paper were undertaken to investigate this problem. Scent tibial plaques have been examined in rare intermorph females determined by a critical photoperiod switching[8], bearing mixed ovaries in which ovarioles of the vivipara and ovipara type are simultaneously present. The relation between plaque number and ovary structure has been studied to test a possible hypothesis of ovarian mediation in scent plaque control.

Material and methods. Experiments were performed on a strain of *Acyrthosiphon pisum* (Harris) reared parthenogenetically under stable laboratory conditions (19 °C, 16 h of light/day) on *Vicia faba* seedling plants.

Oviparous females were obtained from viviparous mothers subjected to short day conditions (8 h of light/day) from 9 days prior to birth. Oviparous/viviparous intermorphs were sought

In table 2 the counts of scent plaques and vitellogenic eggs in the experimental intermorphic females are listed. The figure plots the number of tibial plaques against that of vitellogenic eggs. They are significantly correlated ($p < 0.01$).

The role of JH in regulating the tibial plaque morphogenesis has been assessed by the observed reduction of scent plaque number after JH application[5-7]. These experiments involved application of JH mimics to presumptive ovipara producers. The effect was observed on the F_1 progeny. Intermediate hind tibiae of the treated aphids are very similar in appearance to those of the photoperiod induced intermorphs as obtained in the present experiments[7].

Regarding to the results of JH application experiments 2 remarks may be made: 1. The stage of sensitivity to the hormone does not coincide with that of scent plaque morphogenesis (early adult life) but is coincident with that of ovary differentiation towards viviparous or oviparous development (late embryonic stage). 2. Concomitantly with the reduction of tibial plaque number ovary modifications towards viviparous development resulting in viviparous or mixed rather then oviparous ovaries take place in most of the treated aphids.

It therefore seems reasonable to suppose that JH does not act directly on the scent plaque morphogenesis but rather has a primary action on the ovary. The results obtained here, in which a correlation between the type of ovary development and the plaque number has been found in an aphid without any external hormone application, supports this idea and allows us to postulate an ovarian control of the morphogenesis of the pheromone-releasing organs of aphids.

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