Artificial Fertilization in the Turnip Sawfly, *Athalia rosae ruficornis* Jakovlev (Tenthredinidae, Hymenoptera), by Sperm Injection

Masami SAWA[1] *and Kugao OISHI*[1,2]

[1] *Division of Science of Biological Resources, Graduate School of Science and Technology, Kobe University, Nada, Kobe, Hyogo 657, Japan*

[2] *Department of Biology, Faculty of Science, Kobe University*

We have previously shown that mature unfertilized eggs explanted from adult females of *Athalia rosae* (Tenthredinidae, Hymenoptera) can initiate parthenogenetic development if given various physical or chemical stimuli (Sawa and Oishi, 1987a, b). In the present report, we describe successful artificial fertilization *in vitro* by means of sperm injection using the same sawfly species.

Unmated females about 7-days old were dissected in 0.15M NaCl. Eggs were removed from the ovary, transferred to a piece of double-stick tape attached to a microscopic slide, blotted by a touch of a piece of filter paper, and placed on the stage of an inverted microscope with phase contrast optics. Unmated males of the similar age were dissected in a medium with various concentrations of NaCl or in distilled water (Table 1). Sperm bundles were gently squeezed out from the spermathecae, transferred to a depression slide with a fresh medium, and placed on the microscope stage. Glass injection needles were made by pulling 25 μl micropipets (Microcaps, Drummond). The needle was connected to a "joy-stick" micromanipulator attached to the microscope. Flushing sperm bundles several times in and out of the needle to disperse the sperms in the medium, up to two dozen of sperms were injected into each egg at the anterior pole.

Table 1 Results of sperm injection into unfertilized eggs.*

Sperm suspension medium	Genotypes of: sperm		No. of eggs injected egg	No. of larvae hatched	Nos. pupated	Pupal phenotype ♀		♂	
						yfb/+	+/+ or *yfb*/*yfb*	+ or *yfb*	*yfb*/+
Exp. 1 0.15M NaCl	*yfb*	+/+	1250 (100)	–	222 (17.8)	1 (0.1)	3	216	2
Exp. 2 0.10M NaCl	+	*yfb*/*yfb*	180 (100)	63 (35.0)	44 (24.4)	2 (1.1)	0	42	0
Exp. 3 0.01M NaCl	+	*yfb*/*yfb*	220 (100)	139 (63.2)	54 (24.5)	14 (6.4)	1	39	0
Exp. 4 distilled water	+	*yfb*/*yfb*	199 (100)	100 (50.3)	61 (30.7)	19 (9.5)	0	42	0

*Mature eggs dissected from unmated females were injected with sperm suspended in a medium with an indicated NaCl concentration. Results for Exps. 1-4 are each the total of several replications.

We took advantage of a pupal color mutant, yellow fat body (*yfb*), with which we can recognize the phenotypes *yfb*/*yfb*, *yfb*/+, +/+, *yfb* and +, in order to provide convincing proof of successful *in vitro* fertilization. If injection is successful, diploid females heterozygous for the color mutant, *yfb*, would develop, whereas haploid males would develop in the failures.

In Exp.1, 1250 eggs from wildtype (+/+) females were injected with sperm from *yfb* males suspended in a medium with 0.15M NaCl. Of these, 222 developed to the mid-pupal stage, almost all of which became adults. Of the 222 mid-pupae examined, 216 were parthenogenetic haploid (+) males. Six exceptional individuals were obtained: one female with the color of the heterozygote *yfb*/+, three wildtype-colored (+/+) females, and two males having the color characteristic of heterozygous *yfb*/+ females (Table 1). The single female with the color of heterozygote for *yfb* aged for a week was dissected and her mature unfertilized eggs were parthenogenetically activated. These eggs developed as either *yfb* (30) or wildype (25) haploid males, indicating that this female derived from a fertilized egg. The three diploid wild-type females probably resulted from spontaneous thelytokous reproduction which takes place in this species. Our explanation for two apparently haploid males with coloration chracteristic of heterozygotes is that they are chimeras (*yfb* ↔ +) in which both egg nucleus and sperm nucleus separately participated in development.

Attempts to increase the rate of fertilization was made. Sperms were suspended in a medium with various concentrations of NaCl and in distilled water (Table 1, Exps. 2-4). A highest fertilization rate was obtained when sperms were suspended in distilled water, where about 10 % of the injected eggs developed as heterozygous *yfb*/+ females.

To our knowledge, this is the first successful attempt of artificial fertilization *in vitro* in insects. The technique presented here should provide a means to investigate the process of fertilization in insects. Immediate interests may be whether the transformation of the sperm head to male pronucleus proceeds in the same manner when different numbers of sperm are injected, and whether the egg cytoplasmic factor(s) responsible for this process is localized to a particular place within the egg architecture.

References

Sawa, M. and K. Oishi (1987a) *Proc. Arthropod. Embryol. Soc. Jpn.*, **(23)**, 29-30.

Sawa, M. and K. Oishi (1987b) *Zool. Sci.*, 4, 1060.