

## Induction of gynogenetic haploidy in oyster *Crassostrea gigas*, using ultra violet irradiated sperms

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Eggs of *Crassostrea gigas* were fertilized with sperms exposed to different doses of UV irradiations by keeping a constant rate of  $121.47 \text{ ergs. mm}^{-2} \text{ sec}^{-1}$  and changing the period of exposure from 4 to 90 sec. Samples of larvae were analysed for chromosomal ploidy, rate of fertilization, developmental and chromosomal abnormalities. The gynogenetic haploid larvae first made their appearance in the 30 and 50 sec group exposed to 3644 and 6074  $\text{ergs mm}^{-2} \text{ UV}$ . The maximum number was found in the 90 sec group exposed to  $10932 \text{ ergs mm}^{-2} \text{ UV}$ .

Chromosomal engineering methods of gynogenesis are applied in aquatic species for developing pure lines<sup>1-10</sup>. Earlier, Goswami<sup>1</sup> has described optimum limits of sperm density and some other basic parameters for inducing gynogenetic haploidy in *Chlamys nobilis*. Present communication reports results of gynogenetic induction experiments conducted on the oyster *Crassostrea gigas* (Thunberg) to find out suitable UV irradiation dose for inducing haploidy.

The oysters *C. gigas* were collected from Nakatsohama beach at Nansei, Mie, Japan in June-July, 1988. Milt and eggs from the male and female specimens respectively were artificially removed and pooled in 2 separate beakers containing seawater. These were diluted to  $40,000 \text{ sperms. ml}^{-1}$  and  $2000-2500 \text{ eggs. ml}^{-1}$  and labelled as "sperm stock" and "egg stock" suspension, respectively.

For gynogenetic haploidy induction, 10 glass petridishes of 10 ml capacity were taken and 0.5 ml of sperm stock suspension was spread in each. These were respectively exposed to UV irradiation doses of  $121 \text{ ergs. mm}^{-2} \text{ sec}^{-1}$  for 4, 8, 12, 16, 20, 30, 50, 60 and 90 sec. Total quantity of UV irradiation exposed to each sample was given in Table 1. Later, 1 ml of egg stock suspension and 8.5 ml of seawater were added to each petridish. After 5 min, eggs were transferred to beakers of 50 ml capacity carrying 40 ml of seawater and maintained in a water bath at  $27.5^\circ\text{C}$ . Larvae were allowed to develop for 24 h. Samples for analysing fertilization rate, chromosomal anomalies and developmental abnormalities were collected at 2, 6 and 24 h after insemination. The other details of methodologies were after Goswami<sup>1,2</sup>.

Gynogenesis, production of animals with

maternal genotype homozygosity, was artificially induced by manipulating chromosomes during cell division cycle at 2 steps. The first included destroying genotype of male gametes before fertilization to get haploidy and the second involved resuming diploidy by giving temperature or pressure shocks or using some chemical metaphase arrests at meiosis-I and II or cleavage I<sup>3,4</sup>.

Gynogenetic experiments were performed on some fish and mollusc species using irradiated sperms. Onozato<sup>5</sup> inseminated chum salmon eggs with sperms exposed to  $10^2$  rad to  $10^{5.8}$  rad of  $\gamma$ -rays of <sup>60</sup>Co. Onozato & Yamaha<sup>6</sup> and John *et al.*<sup>7</sup> respectively, exposed milt of 4 species of salmoniformes and *Labeo rohita* and *Catla catla* to UV irradiations. The former workers experimented with 80 to 14,400  $\text{ergs. mm}^{-2}$  of UV rays to induce haploidy. The latter authors did not mention the exact dose given. Onozato<sup>8</sup> could restore gynogenetic diploidy successfully in rainbow trout, cherry salmon and chum salmon by subjecting eggs to hydrostatic pressure ( $650-700 \text{ kg. cm}^{-2}$ ).

Stiles<sup>9</sup> exposed *Crassostrea virginica* oyster milt to X-ray irradiations (10,000 to 25,000 R). He described fertilization in the lower exposed groups as true fertilization as the sperms with severely damaged chromosomes contributed to the developing zygotes. In the higher groups (200,000 and 225,000 R) parthenogenetically developing embryos were reported. Arai *et al.*<sup>10</sup> studied gynogenesis in abalone by exposing sperms from 168 to 360  $\text{ergs. mm}^{-2}$  of UV irradiations. Allen<sup>4</sup> reviewed the available literature on induced gynogenesis while describing other genetic manipulations also. Goswami<sup>1</sup> used UV irradiated sperms for fertilization in the scallop

Table 1—Fertilization, developmental abnormalities (per cent rates) and types of chromosomal abnormalities noticed in *C. gigas* eggs/larvae fertilized with sperms exposed to various doses of UV irradiation. (Figures in parantheses are number of eggs/larvae/cells actually observed)

*Total UV dose (ergs. mm <sup>-2</sup> )	Fertilization		Developmental abnormalities	Type of chromosomal abnormalities
	% (x)	Deviation d = (x - $\bar{x}$ )		
Control *(zero)	99.80 (90)	7.09	0.30 (2000)	Nil
486	74.00 (50)	-18.71	13.85 (65)	Chromatin stickiness (70), laggards and bridges (3), aneuploidy (1), translocation (1), inversion (1)
972	85.70 (55)	-7.01	20.0 (100)	Fragments (20), aneuploidy (18)
1458	85.71 (70)	-7.0	23.08 (156)	Fragments (26), aneuploidy (13), Dicentrics (7)
1944	86.45 (96)	-6.26	50.00 (96)	Fragments (10), aneuploidy (15)
2430	97.50 (50)	-4.79	73.74 (70)	Fragments (2), aneuploidy (37), Anaphase bridge with fragments (3), dicentrics (11)
3644	99.00 (100)	6.29	95.00 (1000)	Aneuploidy (23), anaphase bridges with fragments (4), peculiar chromosome shapes (12), dicentrics (9), haploidy (12)
6074	99.50 (50)	6.84	98.69 (102)	Dicentrics (4), fragments (8), haploidy (23), aneuploidy (52), anaphase bridge with fragments (4)
7288	99.70 (68)	6.99	88.00 (235)	Dicentrics (6), haploidy (52), fragments (17), aneuploidy (22)
10932	99.70 (100)	6.99	5.46 (440)	Haploidy (80), aneuploidy (16), fragments (6)

\*The samples were exposed for zero, 4, 8, 12, 16, 20, 30, 50, 60, 90 sec respectively at the constant rate of 121.47 ergs. mm<sup>-2</sup> sec<sup>-1</sup>. The total dose was calculated by multiplying constant rate with duration of exposure in seconds

*Chlamys nobilis* and reported gynogenetic haploidy induction in eggs fertilized with sperms exposed to 5700-10260 ergs. mm<sup>-2</sup>.

During the present study on *C. gigas*, UV irradiated sperms exposed to varying doses from 486-10932 ergs. mm<sup>-2</sup> were used in fertilizing eggs to induce haploidy. The data on rates of fertilization and morphological abnormalities and chromosomal abnormalities are given in Table 1 and Fig. 1. Fertilization took place in all samples irrespective of dose of irradiation exposure. The sperms acted as physiological activators of the following processes in the egg as reported earlier by Allen<sup>4</sup>. In the true sense of fertilization as described by Stiles<sup>8</sup> it occurred from 486 to 30 ergs. mm<sup>-2</sup>. Thereafter the haploidy started appearing in the zygotes. But it may be stressed here, that the fertilization in the true sense was infact undesirable in the gynogenetic haploidy induction experiments. It was further observed that the rate of fertilization which was 99.8% in the control unexposed group, showed a sudden decline in the lowest exposure group (486 ergs. mm<sup>-2</sup>) with the

maximum negative deviation from the mean (-18.71, Table 1). This may be due to the sudden shock given to sperms by irradiation which might have reduced motility. In a little longer period of irradiation exposure like the next groups 972-1944 ergs. mm<sup>-2</sup>, the sperms got time to recover from the shock. The data collected on morphological abnormalities showed that though the fertilization rate did not reduce sharply in higher exposed groups, the rate of abnormalities increased till 6074 ergs. mm<sup>-2</sup> exposed group. After which it reduced a bit but showed a severe decline in the 10932 ergs. mm<sup>-2</sup> exposed group. It appeared that the germplasm of the sperms in 90 sec exposed group was severely damaged. The 5.46% of abnormal D-shaped larvae observed may be of the haploid larvae. The presence of large number of normal D-shaped larvae in this sample could not be explained.

Chromosomal analysis made in all samples explained the proper situation. In 4 sec exposed group (Table 1) here fertilization rate was low, the developmental abnormalities were also low.

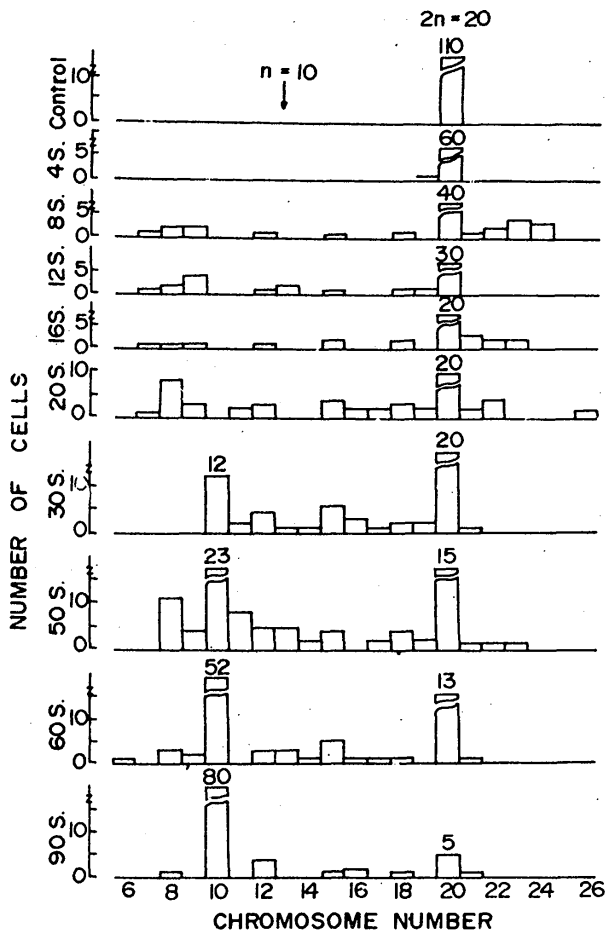


Fig. 1—Number of cells with different chromosome numbers encountered in control and groups exposed to UV irradiations from 4-90 sec, at the rate of  $121.47 \text{ ergs. mm}^{-2} \text{ sec}^{-1}$  (numbers given on top of bars show the number of cells counted in that mode)

Presence of chromatin stickiness, laggards, bridges, translocation and inversions etc. suggested damage on submicroscopic level. The cells with diploid number of chromosomes ( $2n=20$ , Nakamura<sup>11</sup>) were also maximum (Fig. 1). In other doses upto  $2430 \text{ ergs. mm}^{-2}$ , irradiation caused fragmentation of

chromosomes and showed aneuploidy with varying number of fragments of chromosomes included in the zygotes. In the morphological data these abnormalities which were very common corresponded with the abnormal cleavage divisions, abnormal sizes and divisions in morulae, etc.

Chromosomal studies showed the appearance of haploidy ( $n=10$ ) first in the  $3644 \text{ ergs. mm}^{-2}$  exposed group with maximum number seen in the  $10932 \text{ ergs. mm}^{-2}$  exposed group of 90 sec. Increase in number of haploid cells (Fig. 1) was accompanied by a decrease in the number of cells with diploid number of chromosomes. The studies suggested that the gynogenetic haploidy could be induced in *C. gigas*. The haploid larvae survived for 24 h which was the period of experimentation. The optimum dose of irradiation needed for inducing gynogenetic haploidy is in the range  $7288-10932 \text{ ergs. mm}^{-2}$ .

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