FERTILIZATION, MEIOSIS AND CLEAVAGE IN EGGS FROM LARGE MASS SPAWNINGS OF *CRASSOSTREA VIRGINICA* GMELIN, THE COMMERCIAL AMERICAN OYSTER

SHEILA S. STILES and A. CROSBY LONGWELL

U. S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Middle Atlantic Coastal Fisheries Center, Milford Laboratory, Milford, U.S.A.

Received: 2nd May 1972

INTRODUCTION

The development of practical methods for commercial-level hatchery production of the native American oyster, *Crassostrea virginica*, and the opening of commercial hatcheries have led to an interest in its genetic study. The basic breeding system of the oyster has been studied (LONGWELL and STILES 1970). Cytogenetic investigations of its chromosome complement (LONGWELL *et al.* 1967) and a study of normal fertilization and meiosis in its spawned eggs (LONGWELL and STILES 1968*a*) have been carried out.

The following is a report of the regularities and irregularities of fertilization, meiosis and early cleavage in large laboratory-spawned wild populations of the commercial American oyster. Analyses were done on over 1600 eggs from 17 of the large mass-spawned foundation groups of wild American oysters used in establishing the F_1 generation of a two-way selection experiment. The several millions of eggs that were used in the culture of oyster larvae for the first generation of this breeding experiment came from a total of 835 oysters, 462 of which were females and 373, males. Cytogenetic analyses reported here then were so carried out on representatives of a considerable sized cross section of the particular wild populations of the commercial *C. virginica* used.

MATERIALS AND METHODS

Local oysters were wild stock of commercial *Crassostrea virginica* Gmelin from the Long Island Sound, New Haven Harbor area. Non-local stock, also wild,

of the same species came from different geographical areas along the East Coast of North America, ranging from Prince Edward Island, Canada, to the James River, Virginia.

Spawnings were conducted in 1969 during the natural breeding season of the American oyster in Long Island Sound, that is, from mid-June to early August. Spawning and culture methods were those of LOOSANOFF and DAVIS (1963) with some modifications for handling large mass-spawning groups and their mixed gametes.

When temperature of the sea water was raised, usually one or two oysters started spawning and immediately stimulated others to do likewise. The cloudy suspension of spawn was agitated by hand and stirring rod to promote mixing of gametes from different parts of the sink, thereby increasing the chances of all male spawners to fertilize some eggs of several females.

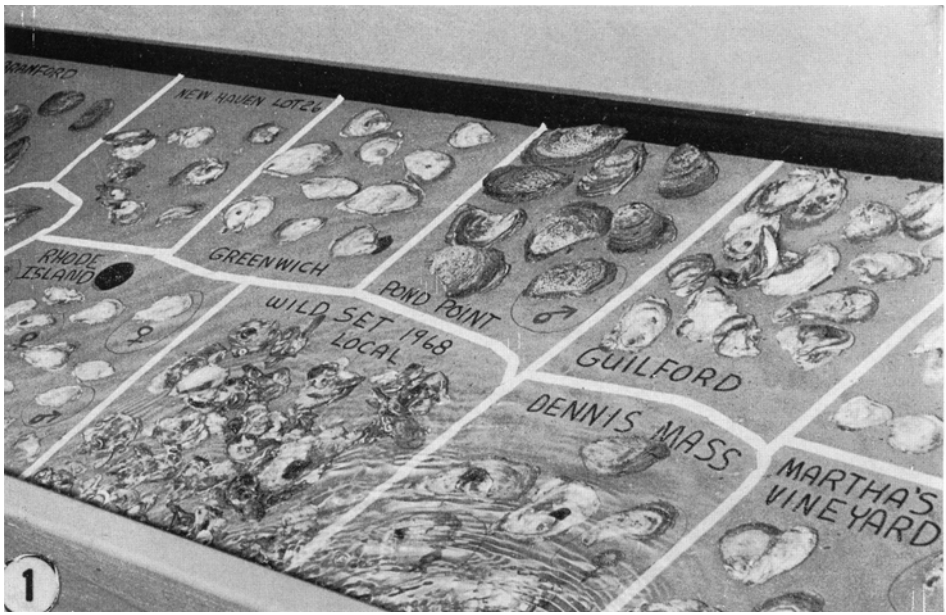


Fig. 1. — Part of a group of wild American oysters set up for mass spawning.

From 21 to 158 animals out of 200 to 400 set up spawned on the different days, approximately one-half to one-third being females on each of the dates. Figure 1 shows some of a group of oysters set up for mass spawning.

From all the mass-spawned groups a total of 668 million eggs at a concentration of 30 eggs/ml were set up for rearing larvae to metamorphosis to the adult form. *

Eggs sampled for cytological examination were fixed and stored in Carnoy's

* Newly metamorphosed larvae are referred to as set or spat.

solution (3 parts 95% ethyl alcohol : 1 part glacial acetic acid). Yolk was extracted from eggs when necessary to visualize the chromosomes and division stages with methanol and chloroform, 1:1, in a micro-Soxhlet apparatus (LONGWELL and STILES 1968*b*).

Fixed eggs and cleavages were stained and lightly squashed in aceto-orcein. A 100 x Zeiss Neofluar phase-contrast objective and Zeiss 12.5 Kpl oculars were used for cytological examination and photography.

RESULTS

The composition of the 17 groups of *Crassostrea virginica* Gmelin on which cytogenetic studies were done, totaling a little over 6000 potential wild oyster spawners for mass release of gametes, varied from all local Long Island Sound oysters to 62% non-locals. Non-locals, also wild *C. virginica*, used with the intention of possibly increasing genetic variability, made up about 18.5% of the total number of oysters actually spawned.

There seemed to be no significant difference between the nine groups with non-locals and the eight without, in per cent eggs fertilized, per cent eggs in abnormal meiosis and cleavage, and per cent development of the eggs to the first larval stage (Table 1). Only one egg in one group of a total of 1624 eggs scored for all groups remained unfertilized.

TABLE 1

Fertilization, polyspermy and development of eggs in variously composed mass-spawned groups of commercial Crassostrea virginica from different geographic areas.

Per cent non-local oysters	Per cent ripe, normal eggs unfertilized	Per cent eggs polyspermic	Per cent ripe, normal eggs in abnormal meiosis and/or cleavage or heteroploid	Per cent development of eggs to first straight-hinge larval stage
0	0	6	17	100
0	0	7	52	55
0	0	16	28	57
0	1	23	29	86
0	0	6	32	100+*
0	0	28	34	100+
0	0	4	21	100+
0	0	11	86	0
14	0	42	11	100+
16	0	22	21	39
23	0	20	41	52
26	0	84	18	35
36	0	26	36	100+
39	0	9	24	100
44	0	44	16	28
57	0	15	10	49
62	0	24	26	100+

* The calculated percentage of over 100% development is attributable to difficulties of obtaining accurate samples of live, actively swimming larvae.

Polyspermy, though present in all groups, occurred two-and-a-half times more frequently in the groups containing non-local oysters, an average of 32% compared to an average of 13% in groups with only locals (Table 1). Although several of the extra sperm nuclei were observed progressing towards a prophase condition, there was no evidence that any actually had contributed their chromosomes to the developing zygotes. This incidence of polyspermy does not correlate with development of eggs to straight-hinge larvae, incidence of abnormal larvae (Table 1), or to setting failure.

Development of eggs to the first larval stage and the incidence of abnormal first-stage larvae, and numbers of set obtained are arranged in Table 2,

TABLE 2

Development of the egg, larval abnormalities and numbers of set obtained for different dates of the mass spawning of variously composed groups of Crassostrea virginica.

Dates of mass spawnings	Per cent development of all eggs to first straight-hinge larval stage	Per cent abnormal straight-hinge larvae	Numbers of spat obtained and eggs set up in culture	
			No. set	No. eggs cultured in millions
June 11	18	4	4	0.45
12	14	1	2	1.8
16	9	1	5	1.8
17	1	0	3	1.8
18	100+*	3	866	3.6
19	48	10	11	3.6
20	100	0	1910	4.0
23	100+	3	1825	3.6
24	55	2	1165	3.6
25	48	1	650	3.6
26	57	14	1513	3.2
27	28	2	47	3.6
July 8	35	0	0	2.5
10	86	6	0	3.6
11	100+	1	0	3.6
15	100+	0	0	3.6
22	49	0	0	3.6
23	100+	0	0	2.7
28	39	3	0	4.5
29	100	2	0	3.1
30	52	2	0	4.5
Aug. 1	100+	0	0	0.8
1	0	-	-	0.5

* The calculated percentage of over 100% development is attributable to difficulties of obtaining accurate samples of live, actively swimming larvae.

according to date of the mass spawning. Development of eggs to the first, straight-hinge larval stage varied from 0 to 100%, fluctuating widely over the entire period of mass spawning from June 11 to August 7. The incidence

of live abnormal larvae at straight-hinge also varied widely for the different dates with no date-related trend, ranging from 0 to 14 with an average of about 2.5%. Abnormal larvae were no less frequent in groups that gave most of the set. Towards the end of the spawning trials no larvae survived to metamorphose to the adult form. This was so even when the development to the straight-hinge larval stage was as high as for earlier spawnings.

The numbers of non-locals in each group do not correlate with the amount of set obtained.

Abnormalities of fertilization, meiosis, and cleavage and heteroploidy occurred in 10 to 86% of the eggs of the different mass-spawned groups. These cytogenetic abnormalities averaged 30% for the 17 groups (Table 3).

TABLE 3

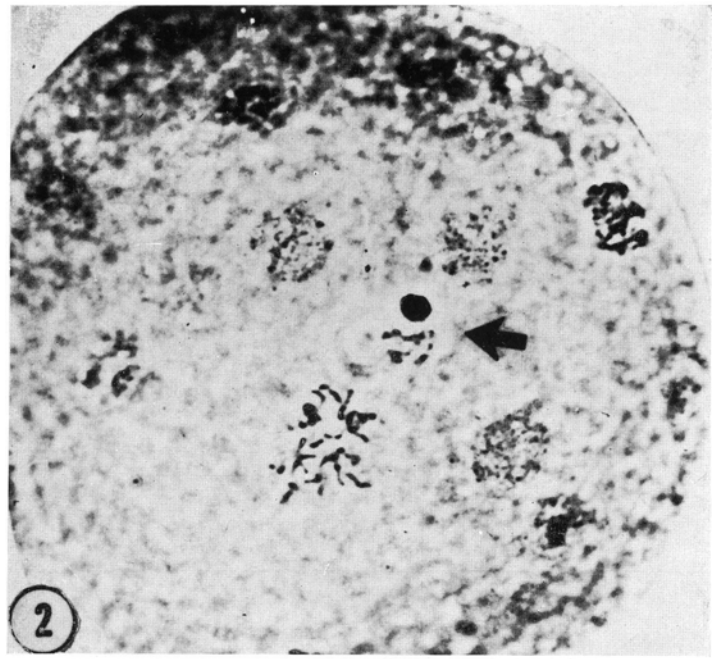
Chromosome, division and nuclear abnormalities in the eggs of variously composed mass-spawned groups of Crassostrea virginica.

Per cent non-local oysters	No. eggs scored	Total per cent eggs with abnormalities	Per cent eggs heteroploid	Per cent eggs with abnormalities of meiosis	Per cent eggs with cleavage abnormalities	Per cent eggs with abnormal cleavage nuclei
0	100	17	8	8	0	1
0	109	52	16	26	3	7
0	105	28	8	18	4	0
0	117	29	11	14	4	2
0	104	32	10	16	2	4
0	114	34	17	9	8	0
0	100	21	5	14	2	0
0	28	86	43	47	14	0
14	100	11	5	2	1	3
16	104	21	8	8	1	5
23	102	41	21	32	0	1
26	112	18	8	6	4	0
36	100	36	17	12	4	3
39	33	24	18	6	0	0
44	107	16	11	12	0	0
57	88	10	1	5	0	5
62	101	26	7	6	1	12

Again, inclusion of non-locals in the population did not appear to be simply related to the occurrence of these abnormalities.

From 1 to 43% of the eggs of the various mass spawnings were heteroploid, the average of the 17 groups being about 12%. Haploids accounted for about 6% of all the heteroploids; polyploids, for about 1.5%; hypodiploids, 1.5%; hyperdiploids, 1.5%; chromosome mosaics, 1.5%. Fig. 2 is an example of a normal diploid, early cleavage egg with interphase nu-

clei, mitotic groups and two normal polar body nuclei. A haploid cleavage egg is shown in Fig. 3.



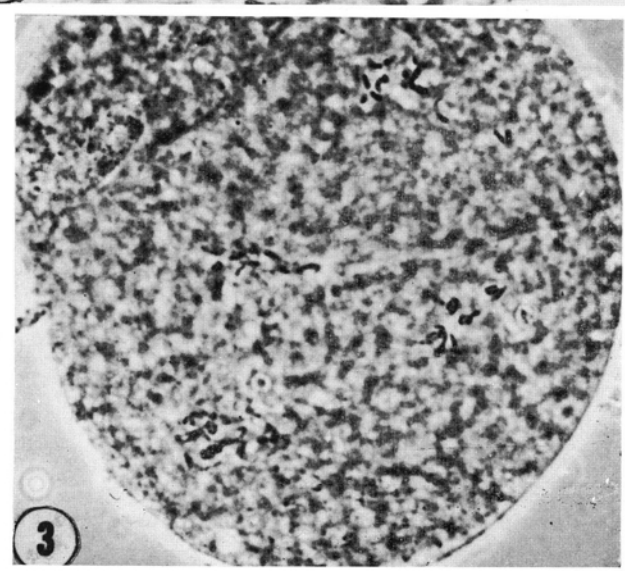


Fig. 2. — Early cleavage egg of the American oyster showing cleavage nuclei, mitotic groups and two normal polar body nuclei. Arrow points to polar body nuclei.
Fig. 3. — An unfertilized parthenogenetic egg of the American oyster in cleavage as a haploid with only 10 chromosomes instead of the $2n$ number of 20.

Meiotic disturbances occurred in 2 to 47% of the eggs in the several mass-spawned groups of eggs, the average of all the groups being about 14%. Disturbances of the cleavage mitoses ranged from 0 to 14%, and averaged about 3%, considerably less than meiotic disturbances. Abnormal-appearing cleavage nuclei occurred in 0 to 12% of the eggs of the several groups, and averaged 2.5%. These nuclei were pale, pyknotic, diffuse or vacuolated, or otherwise of strikingly abnormal appearance.

Fragmentation and bridging of the chromosomes at meiosis indicated that as many as 10% of the eggs could have carried chromosome rearrangements. Some of this bridging, though, must have been due simply to a metabolic or environmentally caused chromosome stickiness.

DISCUSSION

Compared to the frequent failure of a considerable percentage of normal, ripe eggs to be fertilized in laboratory crosses of one male with one female *C. virginica*, the success of fertilization in the mass spawnings is striking. In one series of single diallele crosses of this oyster, using a limited amount of sperm, as many as one-third of the eggs failed to be fertilized (LONGWELL and STILES, unpublished data). To some degree, the greater fertilization successes in the mass spawnings may be attributable to the large selection of gametic types. There is some evidence for gametic cross incompatibility genes in this species, which prevent crossing of the gametes of close relatives (LONGWELL and STILES 1972; LONGWELL and STILES, in press).

Gametic incompatibility in the oyster appears to go hand in hand with polyspermy once fertilization is finally achieved in the presence of excess sperm (LONGWELL and STILES, in press). Compatible crosses are not so likely to become polyspermic even in the presence of equally excessive amounts of sperm.

The increased level of polyspermy in mass spawnings, in which there were considerable numbers of non-local oysters from areas outside of Long Island Sound, could be related to problems arising from incipient crossing barriers. Such a barrier may be arising between at least some populations of American oysters from Long Island Sound and from Maine (unpublished data).

The number of sperm has to approach 20 per egg in the American oyster before the incidence of chromosome and division abnormalities increases significantly (unpublished data). In the mass-spawned groups of oysters the average number of sperm per egg did not approach 20.

Aside from some possible adverse effects of polyspermy, it appears

that non-local oysters can be added to local spawning populations without any other damaging effects on the meiosis, early cleavage or larval stages.

Haploid parthenogenesis alone in the mass spawnings must have occurred in 6% of the eggs. By comparison to some single crosses this is rather high, low in comparison with others (unpublished data on some single crosses; LONGWELL and STILES, in press). Spontaneous haploidy occurs in larvae of salamanders with an incidence of 0.2% (FANKHAUSER 1945). Seedlings of maize, *Datura* and *Antirrhinum*, show an incidence of 0.1%, 0.2% and 0.5%, respectively (see FANKHAUSER 1945, for citations). Although the number of haploid eggs scored in the oyster was much higher than this, the number of larvae actually developing from these eggs was most likely much smaller.

The eggs of pelecypod mollusks must tend to be irregular in chromosome number (AHMED and SPARKS 1967; LONGWELL *et al.* 1967). In the mussel, *Mytilus*, 5-10% of the cleavages are aneuploid, and 1% polyploids (AHMED and SPARKS 1970). These figures are very similar to the 12% incidence of heteroploidy found in the mass-spawned eggs of the American oyster reported here. Species hybrids of *Crassostrea* appear to have a great deal of irregularity in chromosome number (MENZEL 1968). No heteroploid adult has been detected, however, in the course of examination of hundreds of oysters over a period of about five years.

Cytogenetic abnormalities in the mass-spawned groups occurred with the same total over-all frequency as in a series of contemporary single crosses of one female by a number of different males (unpublished data).

Evolution can tolerate considerable wastage of eggs and zygotes in a species as the oyster where a single ripe adult female can produce as many as 35 to 65 million eggs. The large numbers of chromosome and early division abnormalities of the oyster egg are striking. This is so even though the far more protected, internally fertilized eggs of mammals are generally known to have numerical chromosome aberrations. The maintenance of stable oyster populations in the past means that less than one egg out of the 35 to 65 million spawned per adult female each year could be expected to develop into a breeding adult. The fact that this invertebrate has existed relatively unchanged since Carboniferous times (YONGE 1960) points to its reproductive success irrespective of its tolerance of so much primitive reproductive inefficiency.

The oyster's zygotic wastefulness, however, assumes importance now because of the decline of oyster populations in modern times due to the activities of man. Heavy larval mortalities, failure of eggs to reach the first straight-hinge stage of larval development and poor quality eggs are all serious

problems in attempts at making hatchery production of oyster juveniles a commercially successful enterprise. Culture success is presently extremely variable. It must depend on a number of environmental vagaries — effecting cytogenetic difficulties, as well as larval mortalities — related in large measure to the quality of the sea water in which the adults are grown, held, ripened and spawned, and the quality of the water in which embryos develop and larvae are reared.

In a series of experiments run weekly over a period of a year with oysters taken from wild beds in Long Island Sound, per cent development to the first larval stage ranged from 0 to 100; survival of the larvae to metamorphosis ranged from 0 to 94%. About 62% of 50 different cultures reached metamorphosis (unpublished data of W. Landers of Milford Laboratory, for Exper. Biology, cited by permission).

Evolution has failed to provide the oyster egg with much of any safeguard against the sea water environment into which the unprotected eggs are spawned, fertilized, and cleave by the millions from each mature female. Eggs of oysters must so provide a highly sensitive assay cell type for genetically damaging pollutants of the estuaries. This and earlier studies (LONGWELL *et al.* 1967; LONGWELL and STILES 1968*a*) show this mollusk to be well suited to cytogenetic study. Already, use has been made of its early-stage larvae to appraise the killing effects of various marine contaminants (DAVIS and HIDU 1969). Such studies could well be combined with ones on cytogenetic effects of various contaminants.

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SUMMARY

A cytogenetic study was made of over 1600 eggs from 17 mass-spawned groups of 835 wild spawners of the commercial American oyster, *Crassostrea virginica.* Composition of the several groups varied from all Long Island Sound oysters to two-thirds from other geographic areas, ranging from Prince Edward Island, Canada, to Virginia. The addition of oysters to the spawning groups from outside of Long Island Sound did not have any adverse effects on rate of fertilization, meiosis, cleavage, development to the first larval stage, or metamorphosis to the adult form. Polyspermy though was increased.

Abnormalities of fertilization, meiosis, cleavage and heteroploidy occurred in 10 to 86% of the different mass-spawned populations.

Evolution must tolerate considerable wastage of eggs and zygotes in a species such as the oyster where a single adult can produce as many as 35 to 65 million eggs. However, this zygotic wastefulness assumes a practical importance now. This is because of the decline of oyster populations due to the activities of man, and because of interest in commercial production of oysters in hatcheries.

The eggs and larvae of the oyster must be adversely affected by any number of sea water pollutants which cause abnormalities of meiosis, fertilization and cleavage, and often kill larvae which do develop from genetically normal zygotes.

The oyster egg is a good assay cell type for cytogenetically damaging marine contaminants.