# GENETICS OF THE AMERICAN OYSTER, *CRASSOSTREA VIRGINICA* GMELIN

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## INTRODUCTION

The development of refined culture techniques for the commercial East Coast American oyster, *Crassostrea virginica,* at the U.S. government's Milford Laboratory (Loosanoff and Davis, 1963), along with the opening of experimental and commercial shellfish hatcheries in the United States, have led to an interest and modest support of genetic research on the oyster (Longwell, 1969; Longtvell and Stiles, 1970). Our growers of C. *virginica,* whether dealing with a wild oyster set in the field or with their own hatchery products, are faced with the dilemma of cultivating an organism which does either exceedingly well producing a superabundance or does very poorly, both for reasons seldom known. Part of the hoped for process of bringing this oyster under some greater measure of control by the cultivator necessitates some information on its genetic and breeding system. This is particularly so for profitable hatchery production and for the best pond culture. Knowledge of oyster genetics would also be of value for the special stocking of decimated beds or for the introduction of stock to previously uncultivated beds in the wild.

## CHROMOSOME BASIS OF C. *VIRGINICA'S* BREEDING SYSTEM

An examination of the male gametogenesis and of the spawned and fertilized eggs of C. *virginica* revealed that its meiotic system, fertilization, and cleavage are of the typical type found in most higher plants and animals (Longwell and Stiles, 1968a). The oyster is not characterized by any anomalous chromosome behavior as, for example, is the honey bee, which would frustrate breeding plans based on successes in higher organisms. Interestingly, the fate of the fertilizing sperm can be followed from the time of its entry into the egg until the fusion of its chromosomes with the chromosomes of the female gamete on the first cleavage spindle (Fig. 1). It should be noted that any cytogenetic examination of the eggs of at least this species of oyster must be preceded by treatment of the eggs with methyl alcohol and chloroform in a micro-Soxhlet apparatus to remove interfering yolk granules (Longwell and Stiles, 1968b).

There are 10 pairs of chromosomes at metaphase I of meiosis in the mature spawned eggs of C. *virginica* (Longwell, Stiles, and Smith, 1967). Genes are linked together then in 10 different groups. At least in the female the chromosomes indicate that there is a small amount of recombination or crossing-over of the genes making for genetic variability without which there can be no improvement by selective breeding. There is no evidence for any sex chromosomes. This is in keeping with the protandric nature of this oyster. See Figure 2.

The chromosomes of C. *virginica* are all short, about the same length, and hardly any of them readily distinguishable from the others on any basis. This makes for difficulty in their detailed study (Fig.3, 4).

Also studied were the chromosomes of the Japanese oyster, C. *gigas;* the Puerto Rican oyster, *C. rhizophorae;* the European flat oyster, *Ostrea edulis;* the U.S. West Coast small Olympia oyster, *O. lurida;* and the horse oyster, O. *equestris.* All of these species of both the viviparous *Crassostrea* genus and of the larviparous *Ostrea* species have the same number of chromosomes as C. *virginica.* Also, insofar as can be discerned, the chromosomes of all these species are metrically and morphologically like those of C. *virginica* (Longwell et al., 1967). Menzel (1968b) in Florida has examined several other species of these two oyster genera. He further found no variation in chromosome number and morphology. At least at the chromosome level then there should be no barrier to the making of fertile hybrids between the species within these two genera or even between these two genera of oysters. No species of the third oyster

genus, *Pycnodonte,* has yet been examined chromosomally probably because of the difficulties of obtaining these noncommercial forms which occur as singles in deep water.

To date, there have been no indications for any chromosome polymorphism in any of the populations of C. *virginica* in Long Island Sound nor in any other population thus far examined from as far north as Prince Edward Island, Canada, to as far south as the State of Virginia. Ahmed and Sparks (1970) may have uncovered a chromosome polymorphism in marine mussels; Staiger (1957) found extensive polymorphism of chromosome numbers in a marine gastropod mollusk; and Battaglia (1970) has reported extensively on genetic polymorphisms in marine copepods. Chromosome polymorphisms aid the population in making rapid adjustments to fluctuations in the local environment.

In contrast to the population, species, and generic constancy of the chromosomes of the oyster, early developmental stages of C. *virginica* are marked by a high frequency of variation in chromosome number (Stiles and Longwell, unpublished data). Fertilized and cleaving eggs from a series of about 15 massspawned groups, with a total of 835 spawning oysters, had an average of about 26% posttertilization genetic abnormalities. Abnormalities of chromosome number were present in 12% of the eggs. It appears that the sensitivity of this oyster egg to all kinds of environmental distresses results in acute effects at the chromosome level. This should make the oyster an excellent assay species for detecting genetically damaging and zygote-destructive pollutants (Stiles and Longwell, in press).

## INBREEDING C. *VIRGINICA,* ITS EFFECTS AND THE SPECIES MATING SYSTEM

Inbreeding to some extent will accompany mass selection in commercial hatcheries. More extreme forms will eventually be used to develop lines for subsequent hybridization in hopes of so obtaining hybrid vigor.

Full-sib crosses of C. *virginica* made over a period of a year gave consistently poor development to the straight-hinge larval stage. An investigation into this revealed that marked fertilization and early developmental failures were occurring in these crosses (Longwell and Stiles. 1973). In 5 of 9 cultures thus far studied carefully, an average of 63% of the sibcrossed eggs remained unfertilized. Only 13% of the eggs of the contemporary between-line crosses or outcrosses to unrelated wild oysters remained unfertilized. Only 3% of cleavages in the sib-crossed eggs were normal. In the controls 70% of the cleavages were normal. Parthenogenesis averaged 10% in the inbreeding crosses and 0.5% in the controls. See Table 1. Some of the inbreeding crosses were characterized by polyspermy. In others there was a degeneration of the one or more sperm that had penetrated the egg.

A second, more extensive series of sib crosses with their contemporary control interline crosses showed essentially the same crossing difficulties. The incidence of ineffective fertilization was higher than in the first series. Only 46% of the fertilizations actually achieved with sibling sperm activated the eggs to normal development, contrasted to 98% when the sperm of nonrelated oysters of other lines was used. Parthenogenesis was also higher; only 7% in the controls but 29% in the sib crosses.

Prolonged fertilization attempts increased the number of eggs fertilized in sib crosses by 20%. It, however, also led to more polyspermy, and there was more degeneration of sperm in the cytoplasm of the eggs. Such late fertilizations seldom seem to be effective.

These crossing barriers are interpreted as meaning -that a strong outbreeding system in C. *virginica* must, at least in some individuals in some populations, be reinforced by a system of gamete cross incompatibility with a basis in genetic factors. This system must operate to prevent the crossing of gametes of closely related oysters with similar genes. Inbreeding is thereby discouraged, and outbreeding promoted. Incompatibility genes are known to be highly mutable. An increased crossability of C. *virginica* full- and half-sibs originating from irradiated gametes so supports this interpretation.

Genetic systems of cross incompatibility preventing inbreeding exist in an estimated 3,000

higher plants (Brewbaker, 1964; Williams, 1964). The only carefully studied case in animals though has been in a marine organism--the hermaphroditic ascidians (Morgan, 1924, 1942a, 1942b). Some of these are self-fertile, others self-sterile, with some variability between geographic races. Other groups having inbreeding incompatibility often show interspecies crossing barriers as well. From the pioneering work of Imai in Japan (Imai and Sakai, 1961) and the later work of Menzel (1968a) we know that such crossing barriers do exist between oyster species.

Some of the difficulties of inbreeding C. *virginica* in the face of gamete cross incompatibility might somehow be compensated for by making practical use of the parthenogenesis induced in the incompatible matings. A variety of physical and chemical agents and other means can be used to overcome these crossing barriers, as done in other organisms.

As for the less intensive, lower level of inbreeding which will accompany mass selective breeding, incompatibility will tend to keep the level of inbreeding lower than would otherwise occur. This will sometimes work in the breeder's favor, other times against him. When too much inbreeding is practiced too fast, fertilization failures should occur.

In a third group of sib-inbred and between-line crosses of C. *virginica,* food and water levels were adjusted every other day to number of surviving larvae (Longwell and Stiles, 1973). For the period 12 to 17 days, survival of the outbreds was 6 times greater than for the inbreds. In a highly fecund outbreeding species as the oyster a large number of defective recessive genes can be expected to be harbored. On becoming homozygous with inbreeding these will increase total mortality. [Battaglia (1970) has found marine copepods to be extremely sensitive to protracted inbreeding, as are perhaps most highly fecund outbreeding marine species.]

Measurements were made on a total of 1,562 larvae of this series and a least squares analysis of variance done. Looking to the means and standard errors which were quite small--0.7 to 3.0%-- differences in larval lengths between the inbreds and outbreds became apparent by day 6 with differences becoming more pronounced as time progressed (Fig. 5).[2]

These measured differences between inbreds and outbreds point to considerable genetic variation in these oyster stocks. Judging from these results alone there is a good basis for expecting improvements in hatchery-produced C. *virginica* by selective breeding.

Considering the high degree of inbreeding depression and the high mortality of inbred larvae thus far encountered, it can be anticipated that commercial breeders of C. *virginica* will probably find it necessary to hybridize highly selected lines for marketing. Certainly they will have to hybridize intensely inbred lines. No spat have yet been obtained from any of the full-sib crosses made at the Milford Laboratory with the exception of some from the irradiated lines. While some portion of this failure to obtain inbred oysters is undoubtedly attributable to the sporadically and unpredictable poor water quality at the Milford Laboratory, much of it represents the severity of the effect of enforced inbreeding of this oyster. This is particularly significant in that the inbreeding cultures were initiated with unusually large numbers of eggs, as compared to the far smaller population numbers available for studies with species of higher organisms.

Imai and Sakai (1961) detected and made some measurements of inbreeding depression in C. *gigas.* Lines of C. *gigas* were lost in the third generation of full-sib crosses.

## SELECTIVE BREEDING OF *C. VIRGINICA*

Quantitative, commercially important traits are controlled predominantly by exceedingly large numbers of either of two types of genes. The effect of one type is additive; the effect of the other is non-additive. It is the additive type that responds to molding and change by selective breeding. The heritability of a trait is a measure of this additive genetic variance as separated from the other type and from the total phenotypic variance. Heritability estimates can be used to predict progress by selection (see formulae in Fig. 6).

Theoretical heritability estimates should be readily obtainable in the oyster in one generation for any number of commercial traits by crossing the divided lots of eggs of several females by several different males. Contemporaneous cultures of such crosses should provide enough full-sib, and maternal- and paternal-half-sib families for an analysis of variance. From the results of this analysis heritability estimates can be derived directly.

This was attempted several times to estimate heritability of growth rate for laboratory-reared C. *virginica* larvae and spat. Unfortunately, in spite of its merits, this method did not prove successful at the Milford Laboratory. This is because these very high larval mortalities experienced at the laboratory so much of the time seldom leave enough contemporary culture families for statistical comparisons. However, in a commercial-scale pilot hatchery at the University of Oregon operating under better environmental conditions heritability estimates for several different characteristics of C. *gigas* were obtained recently (Lannon, 1972). It is also probable that C. *gigas is* more vigorous and easier to handle in artificial culture than C. *virginica.*

In one series of these diallel crosses of C. *virginica* at Milford enough larval families did survive long enough in sufficient numbers to obtain a rough estimate of heritability of larval growth to the larval age of 2 wk. This estimate, 24%, is in the medium-to-low range.

Realized heritability estimates, as opposed to such theoretical heritability estimates, can be obtained from selection experiments. Selection experiments can also tell something about the duration of the response to selection, and about the limits of selection response for different coefficients of inbreeding, and selection differentials. Such experiments would fare better under the variable culture conditions at Milford than the diallel crosses of the theoretical estimates.

A large selection experiment is currently underway for spat and juvenile growth rate of C. *virginica.* It is intended to keep this breeding experiment going at Milford for an indefinite number of selection generations to estimate the duration and extent of response for a particular inbreeding coefficient and selection differential to upward and downward selection for growth rate. It is hoped to keep the experiment going on a scale large enough to provide seed oysters of known genetic background for other projects

A carefully chosen collection of about 6,000 wild *C. virginica* was set up for mass spawning. Some nonlocal C. *virginica* were included in this group with the hope of increasing the base of genetic variability (Fig. 7). Of the approximate 6,000, 835 oysters spawned. Of these spawners, 85% were from Long Island Sound and the rest from areas extending from Prince Edward Island, Canada, to Virginia.

Several million eggs from 835 spawners were cultured, and the resulting larvae reared to metamorphosis. About 8,000 juvenile oysters were obtained.

At the age of l yr a portion of the surviving juveniles was selected on the basis of their size. The population was divided into a large-selected group, a small-selected group, and a nonselected population. The selection differential for the large juveniles was 18%; for the small juveniles, 8%. The first generation has been obtained from mass spawnings of the two selected populations, and from the randombreeding, unselected $F_1$ control population. This first generation from selected parents is currently being selected itself and spawned for the second selected generation of spat.

Larval data collected in the course of rearing these progeny of the first generation to be selected are showing, as did the larvae from a prior spawning of the same animals which failed to give set, a correlation between selection for juvenile oyster size and larval growth rate. This correlation could be a nongenetic one or a significant genetic one.

First realized heritability estimates are being made on measurements of the spat from the first selected generation of parents at 33 days postsetting. Data are not yet fully analyzed. Nonetheless, preliminary calculations indicate the heritability for fast growth to this age to be

high, 93%.

Considering these estimates, and the possibility in the oyster of very high selection differentials, hatchery breeders should be able to improve growth rate of their oysters. This could be done by mass selection without recourse, for some cycles of selection, to family performance records or progeny testing which are time consuming and costly. Problems will probably arise from inbreeding. Commercial shellfish hatcheries seem prone to start selection programs with too few oysters. Because the spawn of a single, excellent cross might fill even a good-sized commercial hatchery, the problem is accentuated.

Selection progress is being made for resistance to the microsporidian MSX disease of C. *virginica* in the U.S. mid-Atlantic states by both natural selection on the wild beds and in a small artificially selected experimental stock (discussion at 63rd Joint Annual Convention between Shellfish Institute of North America, Pacific Oyster Growers Association, and National Shellfisheries Association, June 1971, Seattle, Wash.). Over a period of years the C. *virginica* oysters of Malpeque Bay, Prince Edward Island, Canada, slowly but certainly made themselves resistant to the Malpeque disease through the agency of natural selection (Needler and Logie, 1947).

## HYBRIDIZATION OF C. *VIRGINICA*

Plants and animals are artificially crossbred or hybridized in order to combine in the offspring some of the desirable characteristics displayed by either set of parents. Another purpose of crossbreeding or hybridizing is to utilize the effects of hybrid vigor. In different species, in addition to the general effects of hybrid vigor, there can be an increase in size sometimes accompanied by partial or complete sterility; increased reproductive capacity sometimes accompanied by a reduction in another character; increased environmental range, or ability to live in a range in which either parent is unable to live; greater uniformity among individuals. Often hybrid vigor is concentrated in a critical stage of early life. Unfortunately, there is no way of predicting for any species just how to obtain hybrid vigor. Inbreeding lines might be test-crossed with one another each generation so that selection can be practiced in terms of the potential of the separate lines for producing heterosis on crossing (see Fig. 8).

Hybridization for the sake of combining the desirable genes of two different types sometimes takes the form of upgrading practiced when the overall performance of the import is better than the local, but when the local is superior in some particularly important traits related to local environmental conditions. The initial hybridization is followed by backcrosses to the import with either natural or artificial selection. Another way of combining the characteristics of two types is the introgression of special desirable foreign genes into the local stocks. This is accomplished by backcrossing the hybrid to the local type again with either natural or artificial selection.

Hybridization of C. *virginica* might supply a less sensitive, more vigorous larval form better able to hold up to the vicissitudes of the hatchery. Hybrids could furnish a diversification of the U. S. East Coast oyster crop. It is a fact that the close cultivation of any species facilitates the spread of disease. Diversification can break the wildfire spread of a disease by the interdispersal of resistant types. It can assure some marketable crop when the disease toll of the susceptible type is greatest. Were more known about the genetics of different geographic populations of *C. virginica,* some of the fear might be diminished of experimental transplantation of oyster set to commercial beds badly in need of seed from areas where it occurs in great abundance and goes to waste.

*C. virginica* occurs extensively over an unusually wide range of temperature, from cold to subtropical, along the Atlantic coast from the Gulf of St. Lawrence to the Gulf of Mexico and farther south to Panama, and around the West Indies. There is evidence for a number of physiological spawning races within the species. Accumulated experience of growers of C. *virginica* indicates that these oysters transferred from one region to another often fail to thrive in the new environment. No doubt, as in *C. gigas* (Imai and Sakai, 1961), there are real genetic adaptations to local conditions, as well as as subtle genetically based morphological and

biochemical differences in the different populations (Stauber, 1947, 1950;LoosanoffandNomejko, 1951;Hillman, 1964; Numachi, 1962; Loosanoff, 1969).

Old, well-established subspecies usually have gene combinations so well adapted to the environments they occupy that any new gene combinations created by hybridization will nearly always be less favorable. If, however, hybridization takes place in an unstable environment, some of the vast array of segregates appearing in later generations will very likely be better adapted to the new field environment than any individuals of the parental group. They could also be better adapted to the artificial environment of a commercial hatchery or experimental shellfish laboratory.

Inclusion of nonlocal C. *virginica* in several of the individual mass-spawned groups that made up the foundation stock of the selection experiment already referred to had no measurable adverse effects in respect to percentage fertilization and percentage development of the eggs to the straight-hinge larval stage. Numbers of dead larvae and abnormal larvae at this stage were not increased--just as many larvae reached setting. There was an increase in the incidence of polyspermy, but this seemed to have no adverse effects.

Wild C. *virginica* sampled from approximately 25 different sites--from areas farmed commercially and from nonfarmeo areas, from Prince Edward Island, Canada, to Greenwich, Conn.--are being test-hybridized.

To date, individual hybrid crosses along with appropriate local and nonlocal control crosses have been made between Long Island Sound oysters and oysters from:

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| Prince Edward Island | Martha's Vineyard, Mass. |
| Maine | Niantic, Conn. |
| New Hampshire | Greenwich, Conn. |

Some spat have been obtained from all these crosses. $F_2$ segregates have been obtained from Niantic and Prince Edward Island hybrids. At least these hybrids are fully fertile.

Fertilization records, larval culture histories, and spat records of all these hybrids and control crosses are being studied. Data accumulated up to the present time appear to show to following:

1. Lack of any crossing barriers between Long Island Sound oysters and the populations thus far crossed with the exception of some oysters from Maine which may be developing an incipient crossing barrier.

2. Good performance of all the hybrids as larvae.

3. But outstanding performance of Maine hybrids as larvae and early postsetting spat.

4. A hybrid growth rate of the spat in the first year slower than that of the local controls, but better than that of the nonlocal controls.

5. Extremely poor growth of the older spat of Maine hybrids and of the Maine x Maine controls with total mortality before the second year. Maine hybrids did better though than the Maine x Maine control.

Full fertility was found in some geographic hybrids of *C. gigas*, along with fertility of their $F_1$ (Imai and Sakai, 1961). Generally speaking, the crossbreds had a higher degree of hardiness, as compared to the inbred strains, and a greater adaptation to environmental conditions. Morphological characteristics fell between those of the strains crossed as did growth, weight, index of meat weight, and glycogen content.

Some years ago H. C. Davis of the Milford Laboratory crossed C. *virginica* with C. *gigas* (Davis,

1950). Fertilization took place readily, cell division was normal, and early veliger larvae were obtained. However, they all died before reaching the umbo stage. Imai and Sakai (1961) obtained the same result. They further found both crossing barriers and hybrid inviability in crosses of C. *gigas* with C. *rivularis* and C. *echinata.* More recently, Menzel (1968a) reported obtaining a few spat from the hybrid cross of C. *virginica* with C. *gigas,* and some spat from crosses of other species with C. *virginica.* Interspecies crosses, at least those involving C. *virginica,* in general, though appear difficult to obtain and difficult to culture successfully (Menzel, 1967, 1971). Recently, a cross of C. *virginica* with C. *angulata,* the Portuguese oyster, resulted in 20% of the eggs developing to the straight-hinge larval stage (Stiles, 1973). All of the larvae, most of which were abnormal, died shortly after this. Cytogenetic examination of this interspecies fertilization revealed 35% of the eggs to be unfertilized. Another 10% had a very delayed fertilization. Polyspermy occurred in 35% of the eggs. Sperm nuclei were abnormally large and irregular in 25% of the eggs. Cleavage was irregular in 5%. Nuclei of the early larval tissues were abnormally vacuolated and highly irregular in shape. Adult oysters were reared from the hybrid C. *gigas x C.* angulata made by Imai and Sakai (1961).

Difficulties in obtaining species hybrids of oysters should not be viewed altogether pessimistically. Some of the methods used in plants and other animals to break down gamete cross incompatibility barriers, as in inbreeding, would, no doubt, be useful in accomplishing interspecific fertilization in the oyster. The problem of hybrid inviability might be overcome by crossing large enough numbers of individuals, carrying larger than usual cultures, by crossing different races of the species involved, or with mutagenic agents. If direct hybrids between the desired species are not possible even then or are sterile, the use of a third "bridging" species can sometimes circumvent the barrier.

Currently the only reliable way to achieve pure hybridization in the oyster without any contaminating nonhybrids is to make single crosses of individual oysters spawned separately, a tedious process not commercially practical. This is so since the oyster cannot be sexed until spawning and because the oyster once sexed can reverse its sex. One male spawning in a group of intended female hybrid parents would fertilize all the eggs before the desired hybrid cross could be achieved. This problem could possibly be solved through the use of some sperm inhibiting agents in the mass-spawning population intended for use as the pool of female parents in the mass hybridization.

## EFFECTS OF IONIZING IRRADIATION ON *C. VIRGINICA*

Mutation breeding has been scarcely attempted in economically useful farm animals. This is because of the high cost of culling out the large numbers of individuals carrying the great numbers of lethal or subvital mutations due to the low reproductive rate of mammals. Because of the oyster's tremendously high reproductive rate and the insignificant worth of a single oyster, there would be no such limitations on mutation studies, or breeding with mutations in the oyster.

Some irradiation-mutation studies were initiated in C. *virginica* for the basic information that could be derived, and to determine the radiosensitivity of this mollusk, a member of a group about which there is relatively little such information (Fig. 9) (Longwell, 1969; Longwell and Stiles, 1970; Longwell and Stiles, unpublished data).[3] There is, of course, the probability of practical application of such information in the future. For example, irradiation might have use in sterilizing highly selected strains of a commercial hatchery to increase somatic growth and to prevent competitor companies from breeding the strain. Irradiation might be used commercially to induce parthenogenesis in obtaining instant, onegeneration pure homozygous individuals. A mutant larval form with a larger mouth could increase the types of algae an oyster larva would find acceptable as a food since the larva would then be able to ingest larger sized algal cells.

Gamma rays from a $Cs^{137}$ source extending from 65 to 10,000 R administered to large, old wild adults in 1 hr. and from 220 to 20,000 R administered in 1 and 2 hr to wild spat about 9 mo old were not sufficient to establish an $LD_{50}$ for C. *Virginica.* The lethal lose must be affected by the

season of the year the oysters are irradiated, as well as by size of the oyster and shell thickness. These oysters and spat were irradiated in the spring of the year as they were coming out of their winter dormancy. Germ-line primordia were beginning active mitoses.

Gross cytological study of the gonads of the gamma-irradiated adults revealed that even the maximum dose of 10,000 R did not adversely affect the production of gonadal material. Instead, the treated group as a whole had roughly about 20% more gonadal bulk than did the control group; also, there were fewer sexually undifferentiated oysters in the treated group.

Spawning performance of the irradiated adults was better than that of the controls. Some adult oysters receiving the maximum 10,000 R spawned. However, none of the juveniles receiving more than 8,000 R spawned.

Cytogenetic study of spawned eggs X-irradiated at a rate of 164 R/15 see and then crossed with untreated sperm showed chromosome damage to begin at 500 R. By 2,000 R such damage was pronounced. The highest dose used on the eggs was 4,000 R.

In terms of the number of set produced and surviving for I yr from cultures of 500,000 fertilized eggs, even the lowest dose of 125 R of X-rays administered at a rate of 199 R/10 sec to pooled sperm crossed with untreated eggs had a slight effect. By 2,000 R the number of these spat was reduced appreciably. At 3,000 R there was hardly any, and none at all at doses above this. From 4,000 R on there was a clear, strong drop in percent development of the eggs to some cleavage stage and percent development to the straight-hinge larval stage. There was an increased percent of abnormal straight-hinge or 2-day-old larvae. The highest dose used on the sperm was 10,000 R. See Table 2.

The overall performance of the larval and spat cultures from the $F_1$ of the gamma-irradiated oysters and from the $F_2$ of the irradiated sperm lines has been characterized by an increased incidence of abnormal larvae at the straight-hinge stage, increased mortality at all the early larval stages, heterosis of the surviving larvae, and possibly some heterosis also in the young spat. This extra vigor must be due to the increased genetic heterozygosity resulting from the irradiation. Irradiation lines are also characterized by a greater crossability of sibs, as mentioned earlier, and also by a greater success of the inbreds. Improved crossability of sibs can likewise be attributed to the increased heterozygosity that results from induced mutations and to mutations of the cross-compatibility genes themselves as well. There has been very little morphological change in either the larvae or spat as a result of the irradiation.

Fertilization and early cleavage stages of these crosses have increased polyspermy, more spindle disturbances, more abnormalities of chromosome number, more chromosome rearrangements, abnormal coiling of the chromosomes, and abnormal nuclei. These are all classic signs of irradiation damage to the genetic material.

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[1] Milford Laboratory. Middle Atlantic Coastal Fisheries Center, National Marine Fisheries Service. NOAA. Milford, CT 06460.

[2] Statistical analysis was done by Ruel Wilson of the Biometrical Services, Livestock Research Staff, Agricultural Research Center. ARS. U.S. Department of Agriculture,Beltsville, MD 20705.

[3] Irradiation was carried out at the Brookhaven National Laboratory with the assistance and advice of A. H. Sparrow.

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