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Title: GENETIC COMPONENTS OF VARIATION IN A BAY MUSSEL EMBRYO BIOASSAY

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The contribution of genetic effects to the variation in percent normal development observed in a bay mussel (Mytilus edulis L.) embryo bioassay was determined. A factorial breeding experiment was accomplished in which 9 males were mated with 3 females. Each mating was repeated 10 times, yielding 270 mussel embryo cultures, which were exposed to 0.00085 parts per million of mercury. This concentration was previously determined to be the level causing approximately 50 percent abnormal development in the progeny of the mussels used in the experiments. All matings were replicated in untreated sea water providing 270 control cultures. The treatment replicate and the control replicate represented two separate environments; therefore, a 3-factor analysis of variance was used to estimate the variance associated with male and female parents, and replications.

The range of percent normal development for the non-treated cultures was 1.6 to 86.4 percent with a mean of 33.9 percent. The

percent normal in the treated cultures ranged from 9.2 to 70.5 percent with a mean of 40.6 percent. The sources of variation related to genetic effects and a genotype-environment interaction were found to be significant. The implications of the results for the interpretation of bioassay results is discussed.

Genetic Components of Variation in a Bay Mussel Embryo Bioassay

by

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Lastly, I dedicate this thesis to my friend and former teacher, Dr. Howard C. Day, who has brightly lit the way for two generations of students.

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INTRODUCTION

The embryo of the bay mussel, Mytilus edulis (L.), has been proposed by Dimick and Breese (1965) as a universal bioassay tool because of its wide geographic distribution in the intertidal zones of estuaries, bays and on some ocean shore areas. This organism also fulfills most of the criteria, identified by Woelke (1968), which define a suitable animal for use in bioassays of marine waters.

A mussel embryo bioassay is a static test which consists of placing eggs and sperm into test solutions. If fertilization and development proceed normally, shelled, straight-hinged larvae (veliger) will result within 48 hours. After 48 hours, the number of normal shelled, straight-hinged larvae or abnormal, non-shelled larvae is counted in a sample of 150 larvae from each culture. Relative to the controls, the proportion (percent) of normal larvae in the test solution constitutes a measure of the response to the substance being tested. Results of bivalve bioassays generally are expressed as the EC 50, a concentration of toxicant resulting in abnormal development of 50 percent of the embryos in 48 hours. Brown (1967) reported the mean EC 50 for the effect of sodium pentachlorophenate on mussel larvae as 0.4 mg/l, but noted that in 19 tests of this

concentration, the results ranged from almost no effect (94 percent normal) to almost complete inhibition of normal development (6 percent normal). Many of the sources of this observed variation in bivalve embryo bioassays have been investigated by Woelke (1972). Environmentally related variation in bioassay results arising from experimental technique and the physiological state of the parents are major causes of variation. Variation arising from quantitative genetic effects was examined in the present study. Specifically, the objective of this study was to determine in the embryos of the bay mussel, Mytilus edulis (L.), the proportion of variance in percent normal development under laboratory bioassay conditions that could be attributed to genetic factors. This information was used to test the hypothesis that the variability observed between groups in a bay mussel embryo bioassay is not only of environmental, but of genetic origin.

The experiment described in this study was conducted at the Oregon State University Marine Science Center located on Yaquina Bay near Newport, Oregon.

MATERIALS AND METHODS

General Methods

A test of the stated hypothesis required development of a methodology permitting separation of genetic variation from variation caused by environmental factors. A basic premise of quantitative genetic theory is that the phenotype (P) of an individual is comprised of a genetic component (G) and an environmental component (E) (Falconer 1960). Thus

$$P = G + E .$$

It follows that phenotypic variance (V_P) has a genetic component (V_G) and an environmental component (V_E). The interaction of genotype and environment adds a third source of variation (V_{GE}), such that

$$V_P = V_G + V_E + V_{GE} .$$

Several breeding designs have been described that permit a partitioning of the total phenotypic variance into components ascribable to these factors. One such design, a factorial breeding experiment (Comstock and Robinson 1952) was used in the research described here.

The variable of interest was the percent of the individuals in each family that developed normally to the 48 hour stage when exposed to a low level of toxicant (methylmercuric chloride).

Experimental Animals

After several difficulties in the experimental techniques were resolved, spawning condition had declined in local stocks of mussels and sexually active individuals could not be found. Mussels from several sites along the Pacific coast were examined for spawning condition and a winter spawning stock of bay mussels was located in Santa Barbara Harbor, California. Several of these mussels were collected, packed in a styrofoam box over ice, and shipped to the Oregon State University Marine Science Center. The mussels arrived within 12 hours after collection in good condition and were put into a concrete sea water aquarium until the experiment could be initiated.

Spawning and Bioassay Methods

To compare the percent of the total number of individuals in each group that developed normally to the 48 hour stage, it was necessary to determine a concentration of mercury (as methylmercuric chloride) that would result in an average percent normal near 50 percent. To meet this requirement, a bioassay was performed prior to the initiation of the factorial breeding experiment.

Methods described by Dimick and Breese (1965) were used to artificially spawn the mussels. Adult mussels were cleaned of

debris and fouling organisms, placed in plastic colanders and stored at 3°C for approximately 14 hours prior to the experiment. This procedure helped to insure immediate pumping by the mussels when they were returned to sea water during the initial spawning process. Sixty mussels were then placed separately in individual finger bowls and covered with sea water at 20°C containing 3 g/liter potassium chloride to stimulate spawning. If no spawning occurred within 45 minutes, the sea water was discarded and replaced with the diluent sea water warmed to 20°C. Mussels generally spawned within 30 minutes to one hour after this change. If no spawning occurred, the entire process was repeated before a new group of mussels was conditioned.

When male spawning had progressed to the extent that the sea water in the finger bowls was quite turbid, the sperm suspensions were decanted into labeled beakers and stored at 3°C. Solutions containing spermatozoa were adjusted to approximately equal concentrations by visual examination with a standard dilution. Only suspensions containing highly active spermatozoa were used in the experiments.

When an adequate number of ova had been spawned, several hundred were collected from each female and transferred into labeled beakers containing filtered, U. V. -irradiated sea water adjusted to a salinity of 25 parts per thousand. The ova were then

suspended by gently blowing into the beaker through a 1.0 ml serological pipette. When the ova were well mixed, a 1.0 ml aliquot was withdrawn and placed in a petri dish. The number of ova in this aliquot was counted under a binocular dissecting scope. Depending on this count, either the volume of sea water was adjusted or more ova were added to the beaker to obtain a final number of approximately 300 to 400 ova per 1.0 ml aliquot. The counts and volume adjustments were continued until two successive counts of 1.0 ml aliquots were within a range of 25 ova or less. The count was then considered final, the numbers recorded and a 1.0 ml aliquot of the suspension was added to each of a series of 25 ml glass vials. At the end of each experiment, three additional counts of ova were made from the suspensions remaining in each beaker. The mean of the five counts was found to be precise within the limits of $\pm$ 10 percent, and was used in the calculation of the percent normal development of the 48 hour old larvae.

Test concentrations of mercury were prepared from a 0.1 g/l stock solution added to filtered, U.V.-irradiated sea water. The stock solution, prepared with distilled water, was made within 24 hours of the beginning of each bioassay. A volume of 20 ml of each concentration used was added to the culture vials prior to the addition of the mussel gametes. Control cultures were prepared with the diluent, filtered, U.V.-irradiated sea water. Three replications were made of the control and mercury treated cultures.

A 1.0 ml standardized (as previously described) aliquot of ova and 0.1 ml (one drop) of standardized sperm suspension were introduced into each culture vial. Thus, fertilization, if not inhibited, took place in the test solutions.

The cultures were kept at $20^{\circ} \pm 2^{\circ}C$ for 48 hours, at which time the vials were capped and preserved by freezing. The cultures were subsequently thawed and total counts were made of the normal veliger larvae which had developed in each vial.

It was concluded from the bioassay that 0.00085 ppm Hg would result in the normal development of approximately 50 percent of the larvae in 48 hours.

Factorial Breeding Experiment

A series of 9 males were mated with 3 females in all possible combinations to produce 27 distinct groups of progeny. Each mating was repeated 10 times, yielding 270 cultures (Figure 1). The entire process was repeated to provide a replicate with 270 cultures as a control.

Spawning methods paralleled those described for the bioassays. Culture containers consisted of 25 ml glass vials labeled as to male parent (1-9), female parent (A, B or C) and repetition (subscripts 1-10) and were arranged in rows of 10, each row comprising the replicate matings of individual pairs. Cultures to be treated with

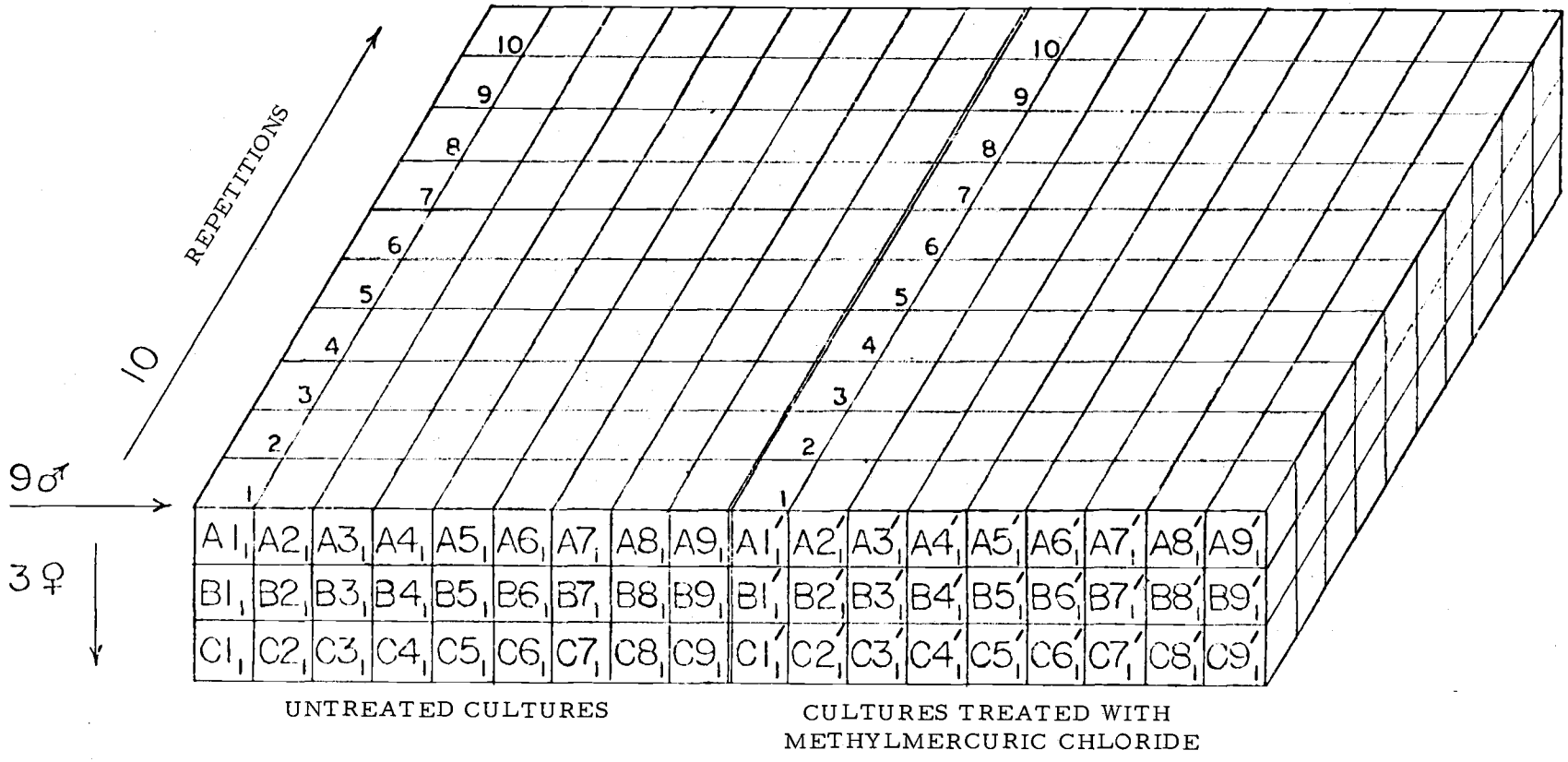

Figure 1. Experimental design of the factorial breeding experiment representing 540 mussel embryo cultures. Letters represent female parents, numbers represent male parents and subscripts denote the repetitions of a mating.

mercury were labeled with red ink, control cultures with black ink.

Immediately preceding the experiment, a solution containing 0.00085 ppm Hg was prepared in filtered, U. V. -irradiated sea water adjusted to a salinity of 25 parts per thousand. A 20 ml volume of the diluent filtered, U. V. -irradiated sea water was added to each of the black labeled vials with an automatic dispensing pipette (1,000 ml flask with a 20 ml volumetric bulb). The mercury treated solution was delivered to the red labeled vials in the same manner.

Spawning procedures were initiated after the vials were filled. Gametes were collected, spermatozoa checked for activity and ova from each female enumerated and standardized as described for the bioassays. Sperm was introduced to all cultures within 15 minutes. The total time from spawning to introduction of sperm was approximately five hours.

The mussel embryos were allowed to develop at 20°C for 48 hours, then were killed and preserved by freezing. The cultures were later thawed and the total number of normal veliger larvae in each vial were counted and recorded. The percent normal development of 48 hour old larvae was calculated as follows:

Let: E = average number of ova in each culture vial (obtained by averaging counts of five 1.0 ml aliquots from each beaker of stock suspensions of ova),

N = total number of normal veliger larvae present in each culture vial 48 hours after fertilization.

Then:

$$\text{Percent normal development} = \frac{N}{E} \times 100 .$$

Data Analysis

In effect, the treatment replicate and the control replicate in this experiment represented two separate environments. Accordingly, a 3-factor analysis of variance (Table 1) was used to partition the variability observed. Male and female parents were considered random effects and the replicate (environments) was considered to be a fixed effect. The general formula for the statistical model was

$$Y_{ijkh} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + e_{ijkh}$$

where Y_{ijkh} = the observation of the h^{th} full sib progeny group of the i^{th} paternal mussel and j^{th} maternal mussel in the k^{th} treatment, μ is the general mean, α_i is the effect of the i^{th} paternal mussel, β_j is the effect of the j^{th} maternal mussel, γ_k is the effect of the k^{th} treatment, $(\alpha\beta)_{ij}$ is the interaction of the paternal and maternal mussels, $(\alpha\gamma)_{ik}$ is the interaction of the paternal mussels and the treatments, $(\beta\gamma)_{jk}$ is the interaction of the maternal mussels and the treatments, $(\alpha\beta\gamma)_{ijk}$ is the interaction of the paternal mussels, maternal mussels and treatments and e_{ijkh} is the remainder term. The important elements in this model are the parent by treatment

Table 1. Format for 3-factor analysis of variance (male and female mussels assumed random; fixed environments)[a].

Source of Variation	Degrees of Freedom	Mean Squares	Expected Mean Squares
Environments	e-1	MS_E	$\sigma^2_W + n\sigma^2_{EMF} + nf\sigma^2_{EM} + nm\sigma^2_{EF} + nmfKe$
Males	m-1	MS_M	$\sigma^2_W + ne\sigma^2_{MF} + nef\sigma^2_M$
Females	f-1	MS_F	$\sigma^2_W + ne\sigma^2_{MF} + nem\sigma^2_F$
Males x Females	(m-1) (f-1)	MS_{MF}	$\sigma^2_W + ne\sigma^2_{MF}$
Males x Environments	(m-1) (e-1)	MS_{ME}	$\sigma^2_W + n\sigma^2_{EMF} + nf\sigma^2_{EM}$
Females x Environments	(f-1) (e-1)	MS_{FE}	$\sigma^2_W + n\sigma^2_{EMF} + nm\sigma^2_{EF}$
Males x Females x Environments	(m-1) (f-1) (e-1)	MS_{MFE}	$\sigma^2_W + n\sigma^2_{EMF}$
Remainder	mfe (n-1)	MS_W	σ^2_W

where: e = number of environments
m = number of male parents
f = number of female parents
n = number of cultures within each mating

[a]After Snedecor and Cochran (1967), p. 368.

interactions $[(\alpha)_{ik}; (\beta\gamma)_{jk}; (\alpha\beta\gamma)_{ijk}]$, which when taken together yield an estimate of the variance due to the genotype-environment interaction.

Prior to analysis, the percentages were transformed to arcsin of the square root of the percent to stabilize the variance (Becker and Marsden 1973). The analysis was accomplished using a variable factor analysis of variance program (Yates 1969) on the CDC 3300 computer at the Oregon State University Computer Center.

RESULTS

The range of percent normal development for the 270 embryos in the non-treated cultures was 1.6 to 86.4 percent with a mean of 33.9 percent. The percent normal in the treated cultures ranged from 9.2 to 70.5 percent with a mean of 40.6 percent. The actual percenrages for all individual cultures are presented in Appendix 1.

F-values (Table 2) were computed from the mean squares associated with each source of variation (Table 3) to determine the significance of each variance (Snedecor and Cochran 1967). To obtain a mean square for the genotype-environment interaction (MS_{GE}), the sums of squares of the parent x environment interaction terms were pooled and divided by the sum of their respective degrees of freedom (Table 4). The F-ratio used to test the null hypothesis that the variance due to a genotype-environment interaction (V_{GE}) = 0 was computed as follows:

$$F_{GE} = \frac{MS_{GE}}{MS_{W}} ,$$

where: MS_{GE} = genotype-environment mean square

MS_{W} = remainder mean square

Table 2. Tests of significance for the important sources of variation in a mussel bioassay. F-values calculated according to Snedecor and Cochran (1967), p. 368.

Source of Variation	F[a]
Males	2.182
Females	25.552**
Males x Females	56.281**
Genotype x Environment	47.535**

[a]The F-value computed for the male effect was statistically significant at the 0.10 level. All other effects were significant at the 0.01 level indicated by a double asterisk (**).

Table 3. Analysis of variance of percent normal development for Santa Barbara mussel embryos from two experimental environments.

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares
Environments	2945.937	1	2945.937
Males	9471.944	8	1183.993
Females	27703.142	2	13851.571
Males x Females	8682.661	16	542.666
Males x Environments	4766.758	8	595.845
Females x Environments	4224.962	2	2112.481
Males x Females x Environments	2925.008	16	182.813
Remainder	4686.809	486	9.642

Table 4. Sources of variance used to compute a genotype-environment mean square.

Source of Variation	Sums of Squares	Degrees of Freedom	Mean Squares
Males x Environments	SS_{ME}	(m-1) (e-1)	--
Females x Environments	SS_{FE}	(f-1) (e-1)	--
Males x Females x Environments	SS_{MFE}	(m-1) (f-1) (e-1)	--
Remainder	SS_{R}	mfe (n-1)	MS_{W}
Genotype x Environment	$SS_{ME} + SS_{FE} + SS_{MFE}$	(fme-fm-e + 1)	MS_{GE}

where: e = number of environments
m = number of male parents
f = number of female parents

DISCUSSION

The main objective of this study was to test the hypothesis that the variability in the test response in a bay mussel embryo bioassay is not only of environmental, but of genetic origin. The sources of variation relating to genetic effects have been shown to be significant. Interpretations of the variance components are presented in Table 5 to identify the sources of genetic and non-genetic variance.

In that the test for a genotype-environment interaction proved to be highly significant, it was indicated that the mussel larvae responded differently, in a genetic context, to the two environments. In regards to similar interactions in plants, Mather (1955) states, "It would appear that different genes are at work or if the same, their whole balance of relative effect has altered." Thus, this interaction implies that control cultures and treated cultures within the same bioassay may have no direct relation to one another in the "control-treatment" sense. This result applies only to the particular conditions of the test and genetic material studied, but it could be important in the interpretation of bioassay results if gene-environment interactions are found to be a common occurrence. Any analysis of bioassay results, which uses a direct comparison of data obtained from control and treatment cultures, may have little meaning. Two such methods of analysis are the "relative percent normal" calculation

Table 5. Interpretations of variance components.

Variance Component	Covariance Estimated	Interpretation
σ^2_M	$COV_{hs(M)}$	1/4 additive genetic variance + paternal effects
σ^2_F	$COV_{hs(F)}$	1/4 additive genetic variance + maternal effects
σ^2_{MF}	$COV_{fs} - COV_{hs(M)} + COV_{hs(F)}$	1/4 non-additive genetic variance
σ^2_W	$\sigma^2_{total} - COV_{fs}$	Remainder of genetic variance + environmental variance + binomial sampling variance + experimental error

where: hs(M) = paternal half-sibs
hs(F) = maternal half-sibs
fs = full-sibs

$(\frac{\text{Treated N}}{\text{Control N}})$ and the "percent net risk" calculation described by Woelke (1972), which is used to correct the bioassay treatment effect for varying abnormality rates in the controls.

The results indicated that additive genetic effects ($4\sigma^2_M$) contributed to the total variance, which implies that selection is acting upon the system. Because of a significant genotype-environment interaction, the presence of this additive genetic variance may have resulted from an adaptive response of the Santa Barbara mussels to an alien environment. Consequently, any selective mortality related to mercury toxicity could not be evaluated. These kinds of experiments may not be appropriate for use with mussels in evaluating genetic changes leading to resistance to toxicants, since the genotype-environment interaction confounds this approach. An alternate, though time consuming, method would be the use of selection experiments (Falconer 1960) to estimate heritabilities for resistance to marine pollutants.

The variance associated with females was highly significant, which indicated that non-genetic maternal effects ($\sigma^2_F - \sigma^2_M$) contribute an important source of variation to the bioassay. Female oysters thermally conditioned for several weeks prior to spawning produced significantly less abnormal larvae (in control cultures) than those with no prior conditioning (Woelke 1972). Mussel bioassays are routinely accomplished with no prior conditioning of the female

parent, which undoubtedly adds an additional non-genetic source of variation to the bioassay results.

The highly significant male-female interaction term indicates that non-additive genetic variance ($4\sigma^2_{MF}$) was a factor contributing to the variance in percent normal development of mussel embryos in the factorial experiment. The significance of this variance lends support to the premise that a mating compatibility factor with a non-additive genetic basis exists in the bay mussel. A similar male-female interaction has been demonstrated to be a significant source of variation in an oyster (Crassostrea gigas) mating system and larval survival in the oyster has been shown to depend to some degree on the combination of parental genotypes (Lannan 1973). Thus, it was concluded that the results of a mussel embryo bioassay depend in part on the specific combinations of parents selected for the test.

BIBLIOGRAPHY

Becker, W. A. and M. A. Marsden. 1972. Estimation of heritability and selection gain for blister rust resistance in western white pine. In: Biology of rust resistance in forest trees. U. S. Dept. Agr. Misc. Publ. 1221, p. 397-409.

Brown, R. L. 1967. The use of the embryonic stages of the bay mussel, Mytilus edulis Linnaeus, as a bioassay tool with special reference to sodium pentachlorophenate. Master's thesis. Corvallis, Oregon State University. 67 numb. leaves.

Comstock, R. E. and H. F. Robinson. 1952. Estimation of the average dominance of genes. In: Heterosis, ed. by J. W. Gowen. Ames, Iowa State University Press. p. 494-516.

Dimick, R. E. and W. P. Breese. 1965. Bay mussel embryo bioassay. Proc. 12th Pacific Northwest Indust. Waste Conf. University of Washington, College of Engineering, Seattle, Wash. p. 165-175.

Falconer, D. S. 1960. Introduction to quantitative genetics. Ronald Press, New York. 365 p.

Lannan, J. E. 1973. Genetics of the Pacific oyster: biological and economic implications. Doctoral dissertation. Corvallis, Oregon State University. 104 numb. leaves.

Mather, K. 1955. Response to selection. Cold Springs Harbor Symp. Quant. Biol. 20:158-165.

Snedecor, G. W. and W. G. Cochran. 1967. Statistical methods. 6th ed. Ames, Iowa State University Press. 172 p.

Woelke, C. E. 1968. Application of shellfish bioassay results to the Puget Sound pulp mill pollution problem. Northwest Sci. 42: 125-133.

_____. 1972. Development of a receiving water quality bioassay criterion based on the 48 hour Pacific oyster (Crassostrea gigas) embryo. Wash. State Dept. Fish. Tech. Rept. No. 9. 93 p.

Yates, T. L. (ed.). 1969. Oregon State University Statistical Program Library. 2nd rev. Corvallis, Oregon State University Department of Statistics. (Pages not numbered)

APPENDIX

APPENDIX I

Percent Normal Development of Mussel Larvae Resulting from the Matings in a Factorial Breeding Experiment*

	A1 (%)	A2 (%)	A3 (%)	A4 (%)	A5 (%)	A6 (%)	A7 (%)	A8 (%)	A9 (%)
1.	46.7	59.0	48.6	50.0	54.3	61.9	60.2	74.5	58.6
2.	56.0	62.1	58.3	55.2	53.1	55.5	54.3	71.0	55.0
3.	51.0	55.2	48.8	46.2	59.3	56.9	59.8	65.7	59.4
4.	48.6	50.9	62.1	40.5	47.1	61.2	55.2	68.8	49.0
5.	30.5	43.8	51.4	46.9	46.7	48.3	45.7	82.4	48.0
6.	40.0	41.7	43.3	45.0	41.2	50.5	48.8	63.8	45.9
7.	41.4	46.7	42.8	47.4	46.2	46.2	46.9	86.4	46.9
8.	31.4	47.1	49.5	38.8	41.9	55.7	42.8	64.8	35.7
9	37.8	47.1	48.8	45.0	52.1	52.9	48.3	70.5	42.9
10.	42.4	44.5	47.9	52.4	50.9	47.4	54.5	80.7	42.9

	A1' (%)	A2' (%)	A3' (%)	A4' (%)	A5' (%)	A6' (%)	A7' (%)	A8' (%)	A9' (%)
1.	51.2	59.2	48.6	64.3	50.2	35.7	39.3	50.2	40.5
2.	66.9	56.7	49.5	43.3	53.1	38.6	45.2	53.3	36.1
3.	52.4	53.8	48.1	51.9	47.4	40.5	35.2	43.4	31.4
4.	59.5	51.7	54.8	55.0	57.4	39.8	38.6	51.2	37.4
5.	51.4	59.3	46.6	46.0	44.3	38.3	41.7	43.8	35.0
6.	57.1	54.0	52.9	45.7	42.9	43.3	41.0	53.8	34.3
7.	60.9	54.3	53.3	51.0	51.2	35.5	52.9	50.9	40.5
8.	47.1	56.2	70.5	42.9	54.8	47.1	45.5	59.5	40.0
9.	46.9	62.1	51.7	66.2	58.1	48.1	50.7	62.6	44.0
10.	47.4	53.6	46.4	48.3	52.9	31.4	48.6	65.0	38.6

	B1 (%)	B2 (%)	B3 (%)	B4 (%)	B5 (%)	B6 (%)	B7 (%)	B8 (%)	B9 (%)
1.	8.1	24.8	14.3	22.9	14.0	48.8	9.4	22.4	35.6
2.	9.4	20.8	16.2	18.3	17.8	49.6	13.5	19.4	35.0
3.	6.2	15.9	9.4	28.3	14.3	44.2	7.5	26.1	28.0
4.	8.6	16.2	10.0	28.8	9.7	53.4	8.9	26.1	23.4
5.	11.0	15.1	14.0	15.4	12.4	42.3	7.0	27.0	27.0
6.	7.8	18.0	8.6	20.5	12.1	38.3	11.3	23.4	35.6
7.	7.3	18.9	9.7	31.5	14.8	49.6	8.1	20.2	27.7
8.	10.2	14.0	9.7	24.2	14.0	48.5	7.3	16.7	26.4
9.	7.8	18.6	6.5	18.9	13.5	44.7	12.9	22.1	24.5
10.	13.5	18.3	9.4	20.8	13.7	41.2	6.7	21.0	26.7

*The letters (A, B, C) represent female parents, the numbers (1-9) represent the male parents and the numbers (1-10) in the left-hand column represent the repetitions of a mating. The symbol (') indicates that the cultures were exposed to mercury.

Appendix I. (Continued)

	B1' (%)	B2' (%)	B3' (%)	B4' (%)	B5' (%)	B6' (%)	B7' (%)	B8' (%)	B9' (%)
1.	9.7	16.4	15.6	23.7	22.9	28.3	16.4	40.2	36.4
2.	9.2	25.6	17.5	17.8	31.5	29.1	12.4	48.0	22.6
3.	10.3	18.9	21.6	23.2	23.4	33.7	14.0	37.2	39.1
4.	11.6	15.6	19.9	29.9	27.8	32.1	22.6	35.6	27.2
5.	15.1	28.0	16.6	28.3	18.9	36.1	17.0	30.7	29.4
6.	11.6	21.6	16.2	25.9	20.2	33.7	21.6	40.2	29.1
7.	15.1	24.2	24.0	26.7	26.1	37.2	19.4	40.4	29.9
8.	10.2	25.1	23.2	23.4	27.0	34.5	17.2	33.2	30.7
9.	15.4	23.2	17.5	28.8	23.7	38.3	21.6	38.0	24.5
10.	10.5	27.2	25.6	24.0	25.1	34.2	19.9	32.1	36.6

	C1 (%)	C2 (%)	C3 (%)	C4 (%)	C5 (%)	C6 (%)	C7 (%)	C8 (%)	C9 (%)
1.	5.2	12.1	37.7	16.6	25.6	59.9	25.8	25.1	46.9
2.	9.9	14.6	32.1	28.2	28.7	45.5	24.0	36.6	66.8
3.	3.6	9.4	35.2	20.6	24.9	51.8	28.7	34.5	57.4
4.	7.4	11.2	32.5	18.8	31.6	51.1	26.0	46.9	60.1
5.	4.7	8.3	35.0	23.1	27.4	47.8	27.1	42.1	65.2
6.	1.8	11.2	34.5	26.0	24.7	55.4	21.7	47.8	59.0
7.	1.6	7.8	30.7	21.3	24.7	47.5	15.0	37.4	61.6
8.	2.2	5.4	28.0	25.6	27.8	48.9	19.3	39.0	62.1
9.	2.5	16.6	30.9	26.9	23.5	49.8	18.4	39.3	55.8
10.	6.0	11.0	28.2	28.4	26.4	49.3	17.9	34.1	61.4

	C1' (%)	C2' (%)	C3' (%)	C4' (%)	C5' (%)	C6' (%)	C7' (%)	C8' (%)	C9' (%)
1.	37.2	38.1	61.4	47.8	51.8	49.3	54.5	44.8	39.2
2.	29.2	33.4	55.8	54.0	57.0	50.7	57.4	48.9	36.1
3.	27.8	28.2	61.4	54.0	61.2	38.9	54.3	57.4	37.4
4.	28.2	34.5	59.2	57.8	57.2	40.4	49.3	51.3	51.1
5.	31.2	41.2	62.3	61.6	65.9	44.6	46.0	50.0	37.2
6.	36.5	32.1	66.6	57.6	58.3	38.1	50.4	55.4	47.8
7.	27.1	35.0	62.3	63.9	57.0	48.3	47.8	58.1	43.9
8.	28.9	39.5	69.0	56.0	57.0	48.4	47.3	52.2	48.4
9.	29.8	32.3	56.0	65.7	54.5	42.4	56.3	59.4	41.0
10.	33.0	42.2	63.9	56.5	50.2	49.6	52.5	43.3	48.0