RACIAL STUDIES IN FISHES. III. DIALLEL CROSSINGS WITH TROUT (*SALMO TRUTTA* L.).

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In previous papers I have summarized the results of some investigations carried out in the Carlsberg Laboratory in Copenhagen with two viviparous species of fish belonging to the genera *Zoarces* and *Lebistes* (*Journal of Genetics*, Vol. VII, 1918, and Vol. VIII, 1919).

It was shown there that the number of vertebrae, fin-rays, etc.—i.e. so-called "racial" characters—are certainly capable of environmental influences, but still primarily determined by internal, hereditary factors[1].

I shall here give an account of some experiments with a third, oviparous, species of fish, whose conditions of reproduction are very favourable to experimental investigation. With this species it has therefore easily been possible to confirm the results previously arrived at with the two viviparous species, and for the same reason it may, presumably, with advantage be used to elucidate generally the difficult questions of the genetic behaviour of quantitative characters.

This species with which I have especially worked is the ordinary trout (*Salmo trutta* L.), with which in this country artificial hatching, applied with a greatly developed technique, is carried on on a large scale.

With a number of trout I perform what I have called *diallel* crossings. It consists in this that *each female is paired with each male.* If for instance there are a males and b females, $a \times b$ offspring-combinations will arise which are all of them different and for which the average values of the character in question are determined by observation. As

[1] Professor R. C. Punnett arrived at a similar result by a statistical examination of the viviparous shark *Spinax niger*, as is evident from an interesting memoir: "Merism and Sex in *Spinax niger*" (*Biometrika*, Vol. III, No. 4, 1904), which was unknown to me, but of which Prof. Punnett has been kind enough to send me a copy.

to particulars of the diallel crossings I refer to a previously published memoir[1].

The experiment here described was carried out during the winter 1918—1919. On the 10th of December 1918 diallel crossings were performed with 7 specimens of trout, viz. 3 males indicated by *x*, *y* and *z* and 4 females (*a*, *b*, *c*, *d*). From this arose 12 different offspring-combinations. The larvae hatched out about April 10 and were killed and preserved on May 15, 1919, when the yolk-sac was just resorbed. The experiment was carried out in the south of Jutland at the "Kongeaa" (Kingsriver) by Mr A. Christiansen. The countings were made by Mr Vilh. Ege, M.Sc.

The character examined was the number of vertebrae, of which the parents exhibited the following:

x 59 *y* 60 *z* 59,
a 61 *b* 59 *c* 57 *d* 58.

For each offspring-combination 50 specimens were examined, in which the number of vertebrae were counted under the microscope, and after this the *average* was calculated for the 50 specimens of each combination.

With notations that indicate an offspring-combination by the symbols of the parents the results were as follows:

TABLE I.

Average number of Vertebrae in Offspring (50 specimens of each combination).

xa	*xb*	*xc*	*xd*
61·14	59·06	58·29	59·03
60·0	*59·0*	*58·0*	*58·5*
ya	*yb*	*yc*	*yd*
61·35	59·22	58·59	59·28
60·5	*59·5*	*58·5*	*59·0*
za	*zb*	*zc*	*zd*
60·65	58·48	57·90	58·55
60·0	*59·0*	*58·0*	*58·5*

In this table the offspring-combinations of a horizontal row have the same father, and those of a vertical row have the same mother.

[1] Johs. Schmidt: "La valeur de l'individu à titre de générateur, appréciée suivant la méthode du Croisement diallèle" (*Comptes Rendus des Travaux du Laboratoire Carlsberg*, Vol. XIV, No. 6, Copenhague, 1919).

The first value under each symbol is the average of the observed numbers of vertebrae in the offspring, the second (in italics) the mean of the parental numbers of vertebrae.

A comparison of the two values shows that in some cases the averages of the offspring and of the parents coincide closely or rather closely (e.g. *xb* and *zd*), while in other cases they differ very much (e.g. *xa*). In other words, there does not appear to be a simple rule connecting the number of vertebrae in the offspring with that in the parents. A closer examination of the values makes it probable that a rule nevertheless exists.

By my previous investigations it has been proved that offspring of the same parents developed under unequal environmental conditions may differ in the number of organs, such as fin-rays, vertebrae, etc. From this it follows that it is necessary to distinguish between the realized, purely *personal* value of a given individual trout—this value would have been a different one, if the individual in question were developed in different environments—and the *generative* value of the same individual, and that is the value which it imparts to its offspring.

It is thus beyond doubt that an individual may have a generative value different from the personal value, and it is possible, nay probable, that in this point we find the cause of the apparent discrepancy above mentioned between the average of the parents and that of the offspring.

At any rate we shall start from the assumption that the average for a number of offspring-individuals closely coincides with the average of the generative values of the parents and inquire whether this supposition does agree with the values arrived at in the experiment. Expressed by formulae our assumption is then that

$$\frac{x+a}{2} = 61{\cdot}14, \qquad \frac{y+a}{2} = 61{\cdot}35, \qquad \frac{z+a}{2} = 60{\cdot}65,$$

where x, y, z, a, etc. indicate the generative value of the individual in question.

By summation of the equations containing x (corresponding to the first horizontal line of Table I) we find

$$2x + \tfrac{1}{2}(a+b+c+d) = 237{\cdot}52.$$

In the same way we find, by summation of the equations containing y and z respectively,

$$2y + \tfrac{1}{2}(a+b+c+d) = 238{\cdot}44,$$

and $$2z + \tfrac{1}{2}(a+b+c+d) = 235{\cdot}58, \text{ etc.}$$

By subtraction we obtain from these equations

$$x-z=0{\cdot}97,$$
$$y-z=1{\cdot}43,$$
$$a-b=4{\cdot}253,$$
$$a-c=5{\cdot}573,$$
$$a-d=4{\cdot}187.$$

Provided our assumption be correct, we have then determined the differences between the generative values of the various individuals, males in one group and females in another.

If we want a measure for the generative values of the single individuals we must fix a starting-point. Let us assume that the generative value of the male y coincides with the personal value of the same specimen, viz. 60, in other words, let us refer all the individuals to those environmental conditions under which the genotype y realizes 60 vertebrae. We then find

$$x=59{\cdot}54,$$
$$y=60,$$
$$z=58{\cdot}57.$$

To find the generative values of the females, e.g. of a, we introduce those values in the equations

$$\frac{x+a}{2}=61{\cdot}14, \quad \frac{y+a}{2}=61{\cdot}35, \quad \frac{z+a}{2}=60{\cdot}65,$$

and we obtain thus three values for a, viz.

$$a_x=62{\cdot}74,$$
$$a_y=62{\cdot}70,$$
$$a_z=62{\cdot}73,$$

the average of which is 62·723.

Proceeding in the same way with the other females we find

$$a=62{\cdot}723,$$
$$b=58{\cdot}470,$$
$$c=57{\cdot}150,$$
$$d=58{\cdot}537,$$

with which we have determined the generative values of all the individuals.

We are now prepared to test our theory by comparing the theoretical values with those obtained by examination of the offspring. By introducing the generative values above calculated into the expressions $\frac{x+a}{2}$, $\frac{x+b}{2}$, etc. we arrive at a *theoretical* value which we want to compare with the average *observed* in the offspring. The results are contained in Table II.

TABLE II.

Comparison of Observed and Calculated Values in the Offspring.

Combination	Observed	Calculated	Difference	Probable Error of observed values
xa	61·14	61·13	+0·01	0·060
xb	59·06	59·01	+0·05	0·040
xc	58·29	58·35	−0·06	0·039
xd	59·03	59·04	−0·01	0·041
ya	61·35	61·36	−0·01	0·056
yb	59·22	59·24	−0·02	0·048
yc	58·59	58·58	+0·01	0·048
yd	59·28	59·27	+0·01	0·051
za	60·65	60·65	0·00	0·054
zb	58·48	58·52	−0·04	0·049
zc	57·90	57·86	+0·04	0·044
zd	58·55	58·55	0·00	0·048

It will be seen that the agreement between the observed and the calculated values is extremely good, also when it is kept in mind that we have had 6—that is 7 minus 1—values of which to dispose freely for calculating the theoretical values. The theory does thus obtain the utmost support obtainable from these experiments.

In Table III are given the personal values of the 7 trout together with their generative values calculated above from the investigation of the offspring. As will be remembered, they were determined on the supposition that all the individuals were referred to environmental conditions under which the genotype *y* realizes 60 vertebrae.

TABLE III.

The Personal and the Generative Values of the 7 Experimental Trout.

Symbols	Personal Values	Generative Values
x	59	59·54
y	60	60 (assumed)
z	59	58·57
a	61	62·72
b	59	58·47
c	57	57·15
d	58	58·54

The table shows that from the personal value of an individual few or no conclusions can be drawn with regard to the generative value. While x and z exhibit the same personal value, viz. 59, the generative value of x is about 1 greater than that of z. On the other hand b and d are nearly identical as regards generative value whereas one of them has realized 1 vertebra more than the other. Between the personal values of a and b and of b and c the differences are in both cases 2, but the differences of the generative values are 4·25 and 1·32 respectively, etc., etc.

Concluding Remarks.

It was mentioned in the introduction that the previous investigations with other species of fish had shown that the number of vertebrae, etc. certainly was influenced by the environment, but still primarily was determined by inheritance. As regards the trout-experiments the fact alone that it has been possible to work, apparently successfully, with the notions *personal* and *generative* values and fix them numerically involves a confirmation of the results of the previous investigations.

Of greater general or rather practical importance is the question, whether the experience gained from the trout-experiments, so favourable in regard to technique, can be made use of in the study of other quantitative characters of greater practical importance in animals and plants. The greater the difference between personal and generative value of an individual the greater the importance, e.g. for the breeder, of a basis which enables him to select animals to breed from, by a systematic valuation of their generative values.

In such cases where it appears that some important quantitative character is inherited in the same way as the number of vertebrae in the trout it may be hoped that the method of diallel crossings with the determination of the average values of the offspring-combinations will prove to be of use.

In most cases, at any rate in the animal kingdom, it will probably be difficult to obtain so great an accuracy in the determination of the generative value of an individual as with the trout. This species is distinguished by a great fecundity and by the great ease and accuracy with which the quantitative character in question could be measured. However, even a less accurate determination would often be of importance, e.g. if the question was of a comparative valuation of some personally identical individuals of a species in which the character studied is highly dependent on environment[1]. I am continuing the work with the method of diallel crossings applied to various species and am especially prepared to test the theory of the method through a study of the subsequent generations.

[1] It is not here the place to discuss at length the difficulties of technical or other nature which the application of the method of diallel crossings may present. I shall only mention that a small fecundity in a species does not necessarily exclude the application of the method, when the character in question may be accurately measured, and, altogether, is inherited in the same way as the character dealt with here. Let us imagine the least possible fecundity, i.e. a brood of one, and let us suppose that we want to compare the generative values of the two males x and y with regard to some quantitative character. We then pair first x and later y with a series of females 1, 2, 3, 4, 5, etc. From this arise two series of offspring-values which may be designated by x_1, x_2, x_3, x_4, x_5, etc. and y_1, y_2, y_3, y_4, y_5, etc. The average of the values $x_1 - y_1$, $x_2 - y_2$, $x_3 - y_3$, $x_4 - y_4$, $x_5 - y_5$, etc. will then afford a measure of the difference between the generative values of the two males, a measure the accuracy of which is inversely proportional to the square root of the number of females paired with the two males; the accuracy may therefore be increased by increasing that number.