CROSS FERTILIZATION AMONG FISHES.

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CONTENTS.

INTRODUCTORY.

In the following pages I wish to record the more important results of my experiments in the cross-fertilization of fishes. These have been in progress more or less continuously since 1898. These experiments were originally undertaken with quite another object in view, namely, for inheritance and cytological studies. A survey of available nearly related forms was made that would successfully hybridize for variation and inheritance studies. Another purpose was the hybridization of forms with different shaped chromosomes, so that the behavior of the latter could be followed in development. It soon developed that the possibility of cross-fertilizing fishes was very much greater than had hitherto been supposed. This led me to seek all possible combinations of species of whatever relationship that happened to be spawning at the same time, and note the possibility and character of impregnation, the development of the hybrids and the fate of developing embryos.

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HISTORICAL.

With one exception, to be noted later, it is possible to impregnate the eggs of any of the species tried, with the sperm of any other species tried, although they belonged to widely separated orders. Isolated instances of equally, or even more distinct crosses have been recorded. Appellöf ('94) made the following crosses among fishes:

Pleuronectes platessa ♀
×
Gadus morhua ♂
Labrus rupestris ♀
×
Gadus morhua ♂

In each of these the species belong to distinct orders. A portion only of the eggs were impregnated. A few showed irregularities in cleavage, and were presumably polyspermic. The European Amphibia have been extensively hybridized by Pflüger ('82) and by Born ('83). The former succeeded in impregnating the eggs of *Rana fusca* with the sperm of both *Triton alpestris* and *Triton taeniatus, i. e.*, an Anuran with a Urodele. The segmentation, however, was irregular so that all the eggs were probably polyspermic. Morgan ('93) succeeded in impregnating the eggs of *Asterias* with the sperm of *Arbacia*. He obtained normal cleavage, the larvæ developing to blastulæ and gastrulæ. His experiments were carefully repeated by Driesch ('98) without result. Mathews ('01) believed Morgan's results were due to parthenogenesis induced by shaking the eggs. Loeb ('03), working with the Pacific Coast Echinoderms, found it impossible under normal condition to fertilize the eggs of *Strongylocentrotus purpuratus* with the sperm of any of the starfish. However, by changing the constitution of the sea water he succeeded in getting impregnations (in some cases 50 per cent.) between *S. purpuratus* ♀ and *Asterias ochracea.* Segmentation was normal; the larvæ developed into blastulæ and gastrulæ, some showing the differentiation of the intestine. Many other experiments in hybridizing fishes have been recorded. These, however, were all between nearly related species, mostly among the domesticated salmonidæ and cyprinidæ. It would not be to the point to pass these in review here. For a good summary of these the reader is referred to Ackermann ('98).

METHODS.

The method of effecting the crosses and the precautions taken to prevent contamination with other sperms, were in all cases essentially the same. The sexes of the same species were kept in separate aquaria. The eggs were expressed into well sterilized watch glasses after which the milt was added. Before adding the milt a sufficient number of eggs were taken from the lot and placed in a fingerbowl of water, as a control. The fertilized lot was also placed in a fingerbowl and allowed to develop there. After the per cent. and character of impregnation was determined and the development well along in segmentation, changes of water sufficiently frequent to insure normal conditions for development were made.[1] All dishes, pipettes, etc., were thoroughly sterilized, first with hot water and then with 95 per cent. alcohol. Notwithstanding the fact that it was found that little danger of contamination existed, the precautions were strictly observed. In not a single instance was there any suspicion that the eggs were not fertilized by the desired sperm.

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[1] I wish to call attention to one defect in the methods of rearing the hybrid eggs. It may be objected that while the rearing of the eggs in the fingerbowl may be satisfactory for Fundulus and some other species it is not normal for a hybrid egg having a sperm from a species that has, for instance, pelagic mode of life during its developmental stages. This unnatural condition may, therefore, in part at least, be responsible for the failures in the development, or even the particular stages at which development ceases. This objection, so far as we know, may or may not be of value. I see no way to avoid this experimental error, since it is not practicable to cater to the demands of one of the parent species without, theoretically at least, infringing on the other. It may be said, however, that many of the species, especially those on which most stress has been laid, have been successfully reared by this method, e. x., all the species of Fundulus, the two species of Sticklebacks, and the two species of Menidia. It is the belief of the writer that this objection may be disregarded.

DESCRIPTION OF CROSSES.

In the following detailed description of the various crosses only such details are included as seemed valuable. Certain of the crosses were kept under observation much more closely than others, and these are more completely considered. It seemed desirable, however, to list the other crosses made, giving brief notes when such seemed worth while. A complete list of all the crosses made is included in Table 9.

Fundulus heteroclitus, female,
×
Menidia notata, male.

This cross was made more frequently and studied more completely than any of the others. A description of the chromosomal behavior has been published by the writer ('94). I included there also a brief description of the development. For the sake of completeness this may be incorporated here. The percentage of eggs fertilized varies from 70 to 93. Actual counts were not made in all the experiments. The percentages in four determinations were as follows:

Experiment	24b	87 per cent.
"	25b	80 " "
"	24b	93 " "
"	126	70 " "

Of the eggs impregnated, approximately 50 per cent. are quite constantly dispermic. Very few are polyspermic so far as can be ascertained by the mode of cleavage. The dispermic eggs never go further than to the close of cleavage. The normally impregnated eggs go through the cleavage stages in a perfectly normal fashion. This is true both for the form and the rhythm of cleavage. In the following table is given a comparison of a lot of hybrid eggs with a lot of normals. The eggs were taken from the same female, fertilized at the same moment and kept under similar conditions. The observations were made at the same time on both lots of eggs and the stage at which each was found was recorded as accurately as possible.

TABLE 1.

TIME OF OBSERVATION.	FUND. × FUND.	FUND. × MEN.
9.10 P. M.., June 26.[1]	In 2 cells.	In 2 cells.
9.40 P. M., June 26.	Beginning 4 cells.	Beginning 4 cells.
10.00 P. M., June 26.	Completion 4 cells.	Completion 4 cells.
10.15 P. M., June 26.	Beginning 8 cells.	Beginning 8 cells.
10.20 P. M., June 26.	Well begun on 8 cells.	Well begun on 8 cells.
10.30 P. M., June 26.	In 8 cells.	In 8 cells.
11.00 P. M., June 26.	Beginning 16 cells.	Beginning 16 cells.
9.00 A. M., June 27.	Well along in segmentation.	Well along in segmentation.
9.00 P. M., June 27.	Well begun on gastrulation.	First trace of gastrulation.
9.00 A. M., June 28.	$\frac{2}{3}$+over the yolk.	$\frac{1}{2}$ or less over the yolk.
3.00 P. M., June 28.	Blastopore closed.	$\frac{2}{3}$ over the yolk.
5.30 P. M., June 28.	Blastopore closed, the embryo long and narrow.	Blastopore closing or nearly losed; embryo much shorter than normal.
9.00 A. M., June 29.	Embryo with optic vesicle.	Blastopore closed, embryo short, no optic vesicle; apparently dead.

[1] Eggs fertilized at 7 P. M., June 26.

From this table it will be seen that the hybrids fall behind the normals in their development. This becomes apparent only in the later stages. In the latter stages considerable irregularity in the rate of development obtains. Usually in a lot of eggs most of which have the blastopore just closed, some eggs may be found that have just entered upon the germ-ring stage. Others may be variously further along. The number of such tardy eggs is usually small. These eggs may stop their development at various stages with consequent shortened embryos and incomplete blastopore closure. In this aborted condition they may live for days, forming pigment both in the embryo and in the yolk. This mass of cells may even develop a heart and ear vesicles. The heart beats for days without, however, handling any blood. From such condition to one where the embryo seems at first to be practically normal there are all stages. The great majority of the embryos die at a condition where the blastopore is closed, the embryo is laid down, though somewhat short, with pigment developed but no heart, eyes, etc.

Some of the embryos, under favorable conditions, develop considerably further. In the more successful of these the yolk becomes highly pigmented with both kinds of chromatophores. The same is true of the embryo. There is an attempt at pattern formation, showing bilateral symmetry but lacking any marked uniformity in the different embryos. A small proportion of the embryos may show only the reddish-brown pigment cells with complete absence of melanaphores. Such embryos are of a strikingly brilliant reddish-brown color. The black pigment may be deposited in the eyes, however. The body of the embryo becomes considerably elongated, though never as long as the normals. The muscle segments are well developed; the vacuolated notocord can be seen and the indications of the vertebral spines can in some cases be made out from a surface view. I have not seen the dorsal and caudal fin-folds developed, except in a very rudimentary way; the pectorals, on the other hand, may be present, and in some embryos are larger than normal. The eyes are at first normally formed, showing as normal optic cups and a well developed lens, and having the normal size. Pigment begins to be deposited much as in the normal, but does not become as abundant. The eye does not keep pace, however, with the normals, so that it finally becomes too small, too slightly pigmented and often lying too low as well as too far forward. The ear vesicle may become very large, appearing as a prominent bulb on either side. The otoliths can be plainly seen. I have seen no indication of a mouth. The brain vesicles form in the earlier stage of the development of these hybrids. Later the brain shows cavities varying in size and regularity, but quite different from the normals. The peri-cardial cavity usually becomes quite large with a volume one-fourth or one-third the size of the whole yolk sphere. The heart becomes often much drawn out. In other cases it is relatively short and may show regions of differentiation. This pulsates vigorously, the wave going in the proper direction. I obtained a single embryo that succeeded in establishing a circulation so that blood was handled by the heart and circulated through the embryo and over the yolk. This circulation lasted for three days, when the vessels became clogged. The heart continued, however, to beat without moving any blood. The usual condition is to have no circulation established. Isolated regions on the yolk show capillaries with colored contents, but no movement of the latter obtains. In the embryo, likewise, lakelets of blood form, a favorite place being in the median ventral part of the tail just posterior to the yolk. I have kept embryos alive for twenty-nine days. The yolk may become reduced to one-half or more in amount. The embryo will not hatch.

Medinia notata, female,
×
Fundulus heteroclitus, male.

The reciprocal of the preceding cross was made four times. The percentage of impregnation does not seem to run as high as in the reciprocal cross. Thus:

Experiment	23b	14 per cent.
"	27	Small per cent.
"	130	88 per cent.

It is probable that the lower percentages of impregnation in experiments 23b and 27 have no significance. The experiments show that under favorable conditions a very high per cent. of impregnation is possible—a condition probably varying but little from the normal.

The condition of dispermy present to such a large extent in the reciprocals does not obtain in this cross.

The rate of development during the earlier stages was the same as that of the normal. The process, however, showed a slowing during the later cleavage stages, as was shown by the normals pretty generally entering upon the germ-ring stage, earlier than the hybrids. Inspection of Table 2. in which the stages of the normals and hybrids are placed in parallel columns, will show that from this point the developmental processes were considerably slowed. Thus, when the blastopore is closed, and the eyes are present in the normals at 4 p. m. 6/15, the hybrids have reached only the stage where the embryo has crept $\frac{1}{3}$ to $\frac{2}{3}$ over the yolk.

TABLE 2.

TIME.	MENIDIA NOTABA × MENIDIA NOTATA.	MENIDIA NOTATA × FUNDULUS HETEROCLITUS.
6.05 P. M., June 12.	Fertilization.	Fertilization.
7.35 P. M., June 12.	Begin. 2 cells.	Begin. 2 cells.
7.35 P. M., June 12.	Close of 2 cells.	Close of 2 cells.
8.50 P. M., June 12.	Begin. 4 cells.	Begin. 4 cells
9.15 P. M., June 12.	Close of 4 cells.	Close of 4 cells.
9.26 P. M., June 12.	Begin. 8 cells.	Begin. 8 cells.
10.08 P. M., June 12.	Close 8 cells.	Close 8 cells.
10.20 P. M., June 12.	Close 16 cells.	Close 16 cells.
10.35 P .M., June 12.	Close 16 cells.	Close 16 cells.
11.05 P. M., June 12.	Close 32 cells.	Close 32 cells.
5.55 A. M., June 13.	Early cleavage.	Early cleavage.
9.00 P. M., June 13.	Germ ring.	Late cleavage and germ ring.
9.00 A. M., June 14.	Germ ring to $\frac{1}{2}$ over yolk.	Early gastrula.
1.30 P. M., June 14.	Begin. gastrula to close of blastopore.	Early gastrula to $\frac{1}{2}$ over yolk.
4.00 P. M., June 15.	Blastopore closed and eyes present.	$\frac{1}{3}$ to $\frac{2}{3}$ over yolk.
2.00 P. M., June 17.		Emb. incompletely formed, optic ves. showing.
2.00 P. M., June 18.		No further along.

The development during the cleavage stages, similar to the reciprocal cross, proceeds normally. It is only in the subsequent stages that the effect of hybridization manifests itself. This shows itself for one thing in the great irregularity of the stages at a given moment. At a time when some of the eggs have proceeded as far as they will go, the greater number of the eggs are in all stages, back to the close of cleavage. This is much more marked than in the reciprocals. It is possible, however, that this is a function of the egg, since even the normals show a considerably greater number of stragglers than do the normal Fundulus eggs. The eggs of this species are evidently less hardy and thus may lend themselves less perfectly to the methods used in rearing them. When development finally ceases the embryos are, for the most part, nearing the closure of the blastopore, the more successful ones showing an embryo with the optic vesicles, but with the body shorter than the normals. The conditions are not essentially different from that described for the reciprocals, except that, as a whole, the development gives out at a somewhat earlier period. This, as already indicated, is possibly due to the less hardy condition of the Menidia egg.

Fundulus heteroclitus, female.
×
Menidia gracilis, male.

Menidia gracilis is distinguished with difficulty from Menidia notata, except in its smaller size. Three experiments were made with this cross.

The percentage of eggs impregnated was as follows:

Ezperiment 119	93 per cent.
" 501	About 5 per cent.
" 503	81 per cent.

In experiment 501 the wet method was employed which probably is responsible for the low percentage. The controls with normal Fundulus eggs showed a correspondingly low per cent. of impregnation.

The number of dispermic and polyspermic eggs was considerably less than in the cross with Menidia notata. In experiment 503 the per cent. was thirteen, about two-thirds of which were dispermic.

The rate of development and the stage at which it stops is similar to that of the cross with Menidia notata. As a whole the number of eggs that successfully effect the closure of the blastopore is greater and the embryos vary considerably less in their lengths, approaching more nearly to the normals. The rudiments of the eye are present. The reciprocal of this cross was not attempted.

In this cross many of the eggs stop their development at the closure of the blastophore with the main axis of the embryo laid down. Many of the eggs continue their development to a varying degree and with varying normality. None of them, however, are developed in a perfectly normal manner. Among these embryos which live for a week or ten days, the most grotesque features appear. The yolk and embryo become pigmented, often very heavily, though not normally. On the yolk the pigment cells may be quite large, or quite finely branched, and they are likely to congregate in certain places, instead of having a distribution such as is found on the normal embryo. A favorite place for such congregation is on the surface between the very large pericardial cavity and the yolk where the pigment cells may densely cover the area. In the embryo the distribution of the pigment may be more or less regular. Thus along the dorsal side two rows may appear in the anterior portion, one on either side, these converging into a single median band running well out towards the posterior end. Both kinds of pigment cells, red and black, are well represented.

The heart is always developed in these embryos and usually pulsates quite vigorously. The excessive development of the pericardial cavity which usually appears as a large clear vesicle—sometimes one-third the size of the yolk—has the effect of stretching the heart out to a great length. As a consequence a curious series of modifications obtain in the different embryos, from a relatively normal heart, although always more or less elongated, to strikingly aberrant conditions, in which the pulsating portion of the heart has become associated with the yolk bordering on the lower portion of the large pericardial cavity, and is a mere mass of cells without apparent structure, and connected with the upper border of the pericardial cavity near the embryo, by an extremely slender protoplasmic thread. No lumen can be detected in either portion and the only effect of the rythmical and vigorous pulsations of the lower yolk portion is to stretch this filament, and pull the yolk upwards so that the latter rocks continually. The usual thing is for the heart to develop a cavity in the interior and the peristaltic pulsations pass in the right direction, *i. e.*, toward the embryo. Out of hundreds of such hearts, many of them relatively normal, which I have examined, I have never seen one carrying blood, certainly not blood containing red corpuscles. In regard to the rest of the circulatory system there is very little to say since it fails to develop. I have never seen any indication of bloodvessels, either in the embryo or in the yolk so far as these could be made out by circlulating blood. There is now and then an embryo that shows what seems, from surface view, a little lakelet of blood. No corpuscles, however, can be seen, and I think they are only accumulations of a pigment of some sort. Nevertheless a considerable portion of the yolk substance is absorbed. This is transferred to the embryo by probably the same method as is employed prior to the development of the vascular system.

The body of the embryo is always much too short and appears heavy. The tail may develop to a considerable length, and in the more successful individuals may show the caudal fin-folds with fine radiations. The body lacks regularity of form and outline. Muscle segments develop, plainly marked off by the brown pigment deposits along their borders. The muscle segments are active, shown by the frequent movements of the tail.

The eyes may be developed to varying degrees, or in many embryos there is no indication of an eye. A quite common condition is the appearance of only a single eye. Some of the embryos show an accumulation of pigment cells either in two patches or one, which because of their location and the fact that they are in rather well-circumscribed patches, probably represent the eye. Two eyes are formed in many. These are always located far forward, so that they seem set into the anterior surface of the head. These may be quite large, well pigmented and showing a lens, or they may be smaller, varying to a condition where merely two small pigment areas are located on the very extreme anterior tip of the pointed head.

The ear vesicle is usually formed. In the place where the vesicle should be there is commonly formed, in the older embryos, an enlarged vesicular structure. This, in some cases, is beyond doubt the enlarged ear vesicle.

The embryos gradually die, but the better formed ones have lived for me for ten days after the normals had hatched.

Fundulus heteroclitus, female,

×

Tautogolabrus adspersus, male.

This cross was made five times. The percentage of impregnation may be almost normal, as shown in experiment 36 in the following table:

Experiment 28b	65	per cent.
" 34b	17	" "
" 36	90	" "
" 102b	35	" "

Practically all of the eggs were normally impregnated, a very few of the eggs fell directly into four and six cells. The rate of development was the same as the normals, until the later stages, when the hybrids fell behind, as shown in Table 3. The table would indicate that the hybrids were a little slower in their cleavage, but this is so slight that no value can be placed on it, considering the difficulty in telling exactly the moment when a new set of furrows begin.

Many of the eggs go far enough to form the embryonic ring and the embryonic shield. The protoplasm continues to spread over the yolk until it is encompassed about two-thirds the way, or nearly closed. The embryo, however, does not form in the shield as it should. I have seen many eggs forming the germ ring and embryonic shield perfectly. The protoplasm continues to grow over the yolk, but the embryo fails to develop perfectly. It is usually much too short and often with the blastopore about closed, there has failed to develop any embryo at all in the shield, the latter remaining a mere mass of cells.

TABLE 3.

	FUNDULUS × FUNDULUS.	FUNDULUS × TAUTOGLABRUS.
Fertilization.	9.30 A. M., July 4,	9.30 A. M., July 4.
Begin. 2 cells.	11.17	11.17
Compl. 2 cells.	11.35	11.35
Begin. 4 cells.	11.50	11.46
Comp. 4 cells.	12.20	12.10
Begin. 8 cells.	12.25	12.15
Compl. 8 cells.	12.45	12.40
Begin. 16 cells.	12.50	12.45
Late segment	12.30 P. M., July 5.	12.30 P. M., July 5.
½ over yoke.	12.00 M. July 6.	12.00 M., July 6.
Blast. closed.	12.00 M. July 7.	Had stopped in previous stage. No embryo differentiated.

Tautogolabrus adspersus, female.

×

Fundulus heteroclitus, male.

This cross was attempted but once and the development followed to the 16-cell stage. Sixteen per cent. of the eggs were impregnated. The rate of development compared with the normals is given in the table below:

TABLE 4.

TIME.	TAUTOGLABRUS × TAUTOGLABRUS.	TAUTOGLABRUS × FUNDULUS.
9.30 P. M., July 4.	Fertilized.	Fertilized.
10.30 P. M., July 4.	Two cells.	Two cells.
10.45 P. M., July 4.	Begin. 4 cells.	Close of 2 cells.
10.50 P. M., July 4.	Well along in 4 cells.	Begin. 4 cells.
11.00 P. M., July 4.	Close of 4 cells.	Well along in 4 cells.
11.10 P. M., July 4.	Well begun on 8 cells.	Close of 4 cells.
11.15 P. M., July 4.	Well along in 8 cells.	Begin. 8 cells.
11.30 P. M., July 4.	Begin. 16 cells.	Close of 8 cells.
11.55 P. M., July 4.	16+cells.	16+cells.

Fundulus heteroclitus, female.

×

Tautoga onitis, male.

The percentage of eggs fertilized in the three experiments made was as follows:

Experiment 104	66 per cent.
" 108	40 " "
" 506	26 " "

It is not probable that with perfectly fresh milt the percentage would be higher. I have observed. the sex products of both sexes in this species materially deteriorate upon confinement of the fish, even for a short time. In experiment 506 good eggs were used and perfectly fresh milt in abundance was used. Practically all of the fertilized eggs are normally impregnated. In experiment 506 every impregnated egg was normal.

The development proceeds in the same manner as in the cross between Fundulus and Tautoglabrus. Most of the eggs form a definite embryonic rim and an apparently normal embryonic shield, but even though they may go to the closure of the blastopore. the embryo is always much too short, never exceeding one-half the normal length. They are mostly shorter than this, a mere thickened mass of cells developed in the embryonic shield. In some cases the blastopore is practically closed, surrounded by a broad embryonic rim with the embryonic shield devoid of any embryo.

Fundulus heteroclitus, female,

×

Gasterosteus bispinosus, male.

The percentage of eggs impregnated may be nearly normal, thus:

Experiment	26d	86 per cent.
"	105d	18 " "

A very few of the eggs may be polyspermic. The details of the development of the hybrid and the normals occur in the table following. It will be noticed that the developmental processes keep apace until the close of cleavage. During the formation of the embryo the hybrids fall perceptibly behind. Most of the eggs go to the closure of the blastopore, although this is accomplished in many eggs only imperfectly. The embryos are largely shorter than the normals. The details of development are itemized in Table 5.

TABLE 5.

TIME.	FUNDULUS × FUNDULUS.	FUNDULUS × GASTEROSTEUS.
2.40 P. M., June 30.	Fertilization.	Fertilization.
5.00 P. M., June 30.	Well along in 2 cells.	Well along in 2 cells.
5.12 P. M., June 30.	Begin. 4 cells.	Begin. 4 cells.
5.57 P. M., June 30.	Still in 4 cells.	Begin. 8 cells.
6.03 P. M., June 30.	Just begin. 8 cells.	Mostly well along in 8 cells.
6.05 P. M., June 30.	Mostly well along in 8 cells.	Well along in 8 cells.
11.00 A. M., July 1.	Toward close of segmentation.	Toward close of segmentation.
12.30 P. M. July 1.,	Late segmentation.	Late segmentation.
1.30 P. M., July 1.	Begin. gastrulation.	Begin. gastrulation.
9.00 P. M., July 1.	½ over yolk.	¼ over yolk.
11.00 A. M., July 2.	⅔ + over yolk.	Mostly ⅓ + over yolk.
12.00 M., July 4.	Embryos formed, brain vesicles, etc.	Embryos formed but no indication of brain vesicles, etc.
12.00 M., July 7.	Eyes formed; tall.	About same as in previous stage.

Gasterosteus bispinosus, female,

×

Fundulus heteroclitus, male.

This cross was made three times, and in one case all the eggs were impregnated. There is the same high mortality usual during gastrulation in these hybrids. Those eggs that laid down the embryo showed the latter very much shortened and the head end very much thickened, many of the embryos appearing to be only head. Rudimentary eyes may form. Details of the development rate, etc., in the normals and hybrids are included in Table 6.

TABLE 6.

TIME.	GASTEROSTEUS × GASTEROSTEUS.	GASTEROSTEUS × FUNDULUS.
1.05 P. M., June 4.	Fertilization.	Fertilization.
4.00 P. M., June 4.	Two cells.	Two cells.
4.30 P. M., June 4.	In 4 cells.	In 4 cells.
4.45 P. M., June 4.	In 4 cells.	In 4 cells.
4.55 P. M., June 4.	Begun on 8 cells.	Begun on 8 cells.
5.15 P. M., June 4.	Close of 8 cells.	Close of 8 cells.
5.25 P. M., June 4.	Begin. 16 cells.	Begin. 16 cells.
6.05 P. M., June 4.	In 32 cells.	In 32 cells.
9.15 A. M., June 5.	Late segmentation. Disk begin. to spread.	Late segmentation. Disk less spread than in normals.
11.40 A. M., June 5.	Germ ring well formed.	No indication of germ ring.
2.00 P. M., June 5.	Germ ring and embryonic shield.	No indication of germ ring.
7.00 P. M., June 5.	½ over yolk.	Germ ring and shield well developed.
6.30 A. M., July 6.	Blastopore closed. Embryo formed.	½ to ⅔ over yolk.
8.00 A. M., June 7.	Embryo developed with eyes, brain, etc.	⅓ over to closure of blast. Embryo short and completely formed.

Fundulus heteroclitus, female, × *Stenostomus chrysops*, male.

In this cross there is always a fairly large proportion of the eggs fertilized. The per cents in four experiments were as follows:

Experiments	103b	70 per cent.
"	106	40 " "
"	122	30 " "
"	508	58 " "

There is usually a considerable proportion di- and poly-spermic. This amounted to 18 per cent. and 20 per cent. in two experiments in which the count was made. The eggs would develop to the closure of the blastopore with the embryo too short though considerably better formed than in the cross Fundulus heteroclitus and Tautogolabrus adspersus. The embryo in some cases may be two-thirds normal length, with the blastopore remaining a rather large oval or slit. Quite a variety of conditions in blastopore closure obtain here, but do not merit detailed description. The relative rate of development for the normals and hybrids is detailed in Table 7.

TABLE 7.

TIME.	FUNDULUS × FUNDULUS.	FUNDULUS × STENOSTOMUS.
10.50 A. M., July 11.	Fertilization.	Fertilization.
11.37 A. M., July 11.	Begin. 2 cells.	Begin. 2 cells.
11.55 A. M., July 11.	Compl. 2 cells.	Compl. 2 cells.
12.05 P. M., July 11.	Begin. 4 cells.	Begin. 4 cells.
12.35 P. M., July 11.	Compl. 4 cells.	Compl. 4 cells.
12.40 P. M., July 11.	Begin. 8 cells.	Begin. 8 cells.
1.00 P. M., July 11.	Compl. 8 cells.	Compl. 8 cells.
1.05 P. M., July 11.	Begin. 16 cells.	Begin. 16 cells.
11.00 P. M., July 12.	Late cleavage.	Late cleavage.
1.25 P. M., July 12.	Well developed germ ring and shield $\frac{1}{3}$ over.	Some begin. of germ ring.
3.30 P. M., July 12.	$\frac{1}{2}$ over yolk.	$\frac{1}{3}$ over yolk.
7.20 P. M., July 12.	$\frac{2}{3}$ over yolk.	$\frac{1}{2}$ over yolk.
8.00 A. M., July 13.	Blast. closed.	Most nearly closed. Embryos too short.
1.00 P. M., July 13.		No further along.

Fundulus heteroclitus, female,

×

Eupomotis gibbosus, male.

This cross was attempted six times, only three of which were successful. The number of eggs impregnated each time was very small. I do not think this was due to the condition of the milt, because an abundance of apparently good milt was used. The dry method was used and the eggs kept in fresh water.

Experiment 7.............................Less than 1 per cent.
" 8............................ " " 1 " "
" 21............................ " " 1 " "

The eggs were followed to about one-third to one-half over the yolk when they stopped development. An embryonic ring and shield were developed, but the protoplasm did not look normal even in earlier stages of gastrulation showing a relatively opaque appearance compared with normal eggs.

The reciprocal of this cross was not attempted owing to a failure to get ripe eggs of the sunfish.

Fundulus heteroclitus, female.
×
Fundulus diaphanus, male.

Fundulus diaphanus was obtained from a fresh water lake, and was crossed many times in both directions with Fundulus heteroclitus. In the present cross I was never able to get a higher degree of impregnation than 80 per cent. Often I failed altogether and usually there was only an occasional fertilized egg—less than 1 per cent. This may have been due partly to the difficulty of getting enough good milt. In one instance I found about one-half of the eggs polyspermic. The eggs develop along in a normal fashion, going somewhat slower in the later stages, and hatch. I have reared some of the embryos for two weeks and they seemed perfectly active and otherwise normal. The eggs were reared in both fresh and sea water.

Fundulus diaphanus, female,
×
Fundulus heteroclitus, male.

When Fundulus diaphanus is used as the female nearly all of the eggs may be impregnated. Thus:

Experiment 6b....................Nearly 100 per cent.
" 13a.................... " 62 " "
" 18a.................... " 91 " "

The eggs are practically all normally fertilized and hatched. These I have reared for twenty-two days after hatching.

Fundulus heteroclitus, female,
×
Cynoscion regalis, male.

The eggs of Fundulus are fertilized as perfectly with the sperm of the Squetegue as with its own milt. Thus:

Experiment 513..............................92 per cent.
" 514..............................90 " "

Only about 2 per cent. of the eggs are polyspermic. A tabulated outline of the development compared with the normal is as follows:

TABLE 8.

TIME.	FUNDULUS × FUNDULUS.	FUNDULUS × SQUETEAGUE.
3.40 P. M., July 17.	Fertilization.	Fertilization.
5.50 P. M., July 17.	2 cells.	2 cells.
6.35 P. M., July 17.	4 cells.	4 cells.
7.25 P. M., July 17.	8 cells.	8 cells.
7.45 P. M., July 17.	Begin. 16 cells.	Begin. 16 cells.
8.10 A. M., July 18.	Late cleavage.	Late cleavage.
12.20 P. M., July 18.	Begin. germ ring.	Begin. germ ring.
7.00 P. M., July 18.	$\frac{2}{3}$ over yolk.	Germ ring; $\frac{1}{3}$ to $\frac{1}{2}$ over.
8.15 A. M., July 19.	Blast. closed; optic vesicles plainly formed.	Blast. closed; optic vesicles poorly formed notocord; somites. Behind normals.
2.15 P. M., July 19.	Optic vesicles and lens; brown parts showing.	Optic vesicles showing; behind normals.
	Hatched.	No further along.

The embryos may continue their development to a stage where the eyes, heart, ear vesicles, tail, etc., are more or less well formed. At this stage they remain alive until about the time that the normals hatch.

None of the embryos are formed even approximately normal. The individual differences are so great that a description of different forms would be of no avail. A brief description of one of the poorer embryos is as follows: The embryo may form a mere mass of cells so far as external appearances go. This embryo becomes pigmented with many pigment cells which are highly branched. It is bilaterally symmetrical in form, but the distribution of the pigment cells only slightly indicates this. In this embryo there was no definitely differentiated heart, but beneath and about one-third the distance back from the anterior end, a mass of cells could be seen regularly pulsating. Just above the heart-mass a vesicle occurred which I take to be the ear. There was no indication of a tail. I repeatedly observed the embryo bend itself from side to side so that there were probably muscle tissues formed on both sides. This particular embryo died nine days after fertilization.

One of the best formed embryos may now be described. Two eyes are formed, although much too small, poorly pigmented, without a lens and set into the anterior surface. Extending out on either side is a large vesicle which is probably an hypertrophied ear vesicle. A pretty well developed tail is shown. Notocord and muscles are developed. A long tubular heart extends across the rather large pericardial sac. The body shows many finely divided pigment cells. These show, in general, a bilateral distribution. Even this embryo is considerably too short. The contractions of the body are vigorous and frequent. The heart beats considerably slower than the normal. This embryo lived until the normals had hatched.

The development of eyes, as in the preceding embryo, is uncommon. There are often pigmented areas which are probably the representatives of this organ, but no definite vesicles or cups. In many of the embryos the anterior end is occupied by enlarged vesicles which is more or less heavily pigmented.

All the embryos are too short, many of them mere short masses of cells without any caudal elongation at all. They are all highly pigmented, the prevailing color being a reddish-brown. These cells are as a rule very finely divided. The dark pigment cells are relatively few in number and are, as a whole, much less finely branched. I have never been able to see any bloodvessels that were carrying blood. In a few instances irregular lakelets, reddish in color, appeared, but I have been unable to detect any corpuscles in them.

In addition to the above crosses it seems worth while to include the other crosses effected. To these much less attention was given so that in a description of them only such points as seem relevant will be given.

Crosses with Gasterosteus bispinosus.

Besides the crosses already described between Gasterosteus bispinosus and Fundulus heteroclitus, the following were attempted:

Gasterosteus bispinosus, female,
×
Apeltes quadracus, male.

In the single attempt to make this cross, only 17 per cent. of the eggs were impregnated. The eggs from a single female were used. It is probable that further attempts with more favorable females would yield a greater per cent. of impregnation. The fertilized eggs were normally impregnated. After the cleavage stages the hybrids fell behind the normals so that while the latter had closed the blastopore, the hybrids had encompassed the yolk about three-fourths of the way. Seven embryos were hatched and were in an apparently normal condition. These were kept alive for four days.

A smaller proportion of the embryos failed to emerge from the membranes. These were helped out but showed the coiled tail so common among fish embryos that seem to have thrived poorly. The hybrids, however, appeared less healthy than a lot of normals that were fertilized at the same time, and kept under the same conditions. These mostly lived three and four days longer. As far as can be judged from this single experiment, it is doubtful whether many of these hybrids, even with the care and proper conditions supplied, could be successfully reared.

Apeltes quadracus, female,
×
Gasterosteus bispinosus, male.

Two tests were made. In one of them 18 per cent. of the eggs were impregnated. The embryos showed the usual slowing in the rate of development after close of cleavage. The development went to the stage of hatching, two emerging but showing little vigor. They died after the second day of emergence. The embryos that failed to emerge, for the most part lived as long as the two which had hatched. The success of this cross is probably the same as that of the reciprocal.

Gasterosteus bispinosus, female,
×
Menidia notata, male.

The eggs of this stickleback are practically all impregnated when placed with Menidia sperm. In the two experiments tried, 100 per cent. and 70 per cent. were fertilized. A small per cent. of these are polyspermic. The development keeps well apace with the normals until toward the closure of the blastopore. The embryo is laid down, the eyes are formed, but the anterior region of body is quite heavy. Pigment forms and the heart is developed. I have never seen fins form in these hybrids. The embryos soon die, owing possibly to the fact that the eggs even normally do not do well in a fingerbowl of water.

Menidia notata, female,
×
Gasterosteus bispinosus, male.

This cross was made but once. All of the eggs were fertilized. The development was followed to the closure of the blastopore. They doubtless developed further since my notes, at this stage, show them to be in a good condition.

Gasterosteus bispinosus, female,

×

Menidia gracilis, male.

In two of the three experiments made with this cross, practically 100 per cent. of the eggs were normally impregnated. The eggs develop at the same rate as the normals until the latter half of gastrulation, when the hybrids fall behind. Most of the eggs form embryos. These are short, with heavy anterior portion. The posterior one-half or one-third of embryo remains quite rudimentary. The anterior enlarged end develops eyes that in the earlier stages are apparently normal. The heart is formed and pulsates. I have kept these embryos alive for five days.

Gasterosteus bispinosus, female,

×

Tautoga onitis, male.

Practically 100 per cent. of the eggs were normally fertilized in the single cross made. The eggs show the usual slowing in development at the close of segmentation. About 50 per cent. of the embryos died at a stage when gastrulation was from one-third to one-half completed. The remainder more or less completely closed the blastopore. The anterior portion of embryo is heavy. No eyes and heart were observed. The embryos died three days after closure of the blastopore.

Gasterosteus bispinosus, female,

×

Tautoglabrus adspersus, male.

Seventy-four per cent. in one experiment and practically 100 per cent. in the other were normally impregnated. The embryos developed more successfully than when Tautoga was used as the male. A large per cent. of the eggs attempted to close the blastopore. The anterior end of embryo was large, eyes and heart were developed but not normally. The embryos lived for five days in this condition.

Menidia notata, female,

×

Tautoga onitis, male.

Eighty per cent. of all the eggs were normally fertilized. The embryos were followed to the closure of the blastopore. The embryos were shorter than normals.

Tautoga onitis, female,
×
Menidia notata, male.

In this cross 60 per cent. of the eggs were impregnated. Some dispermy and polyspermy occurred. There was a heavy mortality at the germ ring stage and subsequently. The ring spread about two-thirds over the yolk in some of them, when owing probably to bad conditions, all died.

Fundulus diaphanus, female,
×
Eupomotis gibbosus, male.

The cross between the fresh water Fundulus and Eupomotis is from the standpoint of impregnation, much more successful than when the egg of Fundulus heteroclitus is used. The percentage of eggs fertilized may be as high as 70 per cent.

Experiment	12	56	per cent.
"	17a	11	" "
"	18b	70	" "
"	22a	23	" "

I have always found a considerable number of imperfect eggs which accounts in a measure for the usual low per cent. of eggs fertilized. A particularly large number occurred in Example 17a. A few of the eggs are polyspermic in each experiment, probably, however, not many more than in the normals, where there may be as many as 5 per cent. polyspermic. The development stops when the protoplasm has spread about one-half over the yolk. Embryonic ring and shield are formed, but very little evidence of embryonic differentiation being shown in the shield. The protoplasm looks granular and opaque instead of clear, as in the normals.

Opsanus tau, female,
×
Fundulus heteroclitus, male.

In the one experiment made, twenty-one out of thirty-seven eggs were found in the 2 and 4-celled stage eight hours and twenty-five minutes after fertilization. A few of the eggs were polyspermic. They were followed to later cleavage.

Opsanus tau, female.

×

Tautogolabrus adspersus, male.

One out of thirteen eggs was impregnated in the single experiment. The rate of cleavage was the same as in the above cross.

Opsanus tau, female,

×

Menidia notata, male.

Thirteen out of seventeen eggs were impregnated. None of the eggs were polyspermic. The cleavage rate was the same as the Opsanus X Fundulus cross listed above.

The reciprocals of the above named three crosses with Opsanus tau eggs were attempted but without success. This was doubtless due to the unripe condition of the Upsanus tau milt, since I was also unsuccessful in obtaining normals.

In addition to the crosses detailed above the following were also effected (See Table 9):

Fundulus heteroclitus ♀ × Apeltes quadracus ♂.
Tautoga onitis ♀ × Tautogolabrus adspersus ♂.
Tautogolabrus adspersus ♀ × Tautoga onitis ♂.
Coregonus clupeiformis ♀ × Argyrosumus artedi ♂.
Argyrosomus artedi ♀ × Coregonus clupeiformis ♂.
Cristivomer namaycush ♀ × Salvelinus fontinalis ♂.

Table 9.

Cross.	Family.	Order.	Number of Crosses Made.	Per Cent. of Impregnation.	Remarks.
Fundulus heteroclitus ♀ Fundulus diaphanus ♂	Pœciliidæ Pœciliidæ	Haplomi Haplomi	8	0–80	Hatched.
Fundulus diaphanus ♀ Fundulus heteroclitus ♂	Pœciliidæ Pœciliidæ	Haplomi Haplomi	10	62–100	Hatched.
Fundulus heteroclitus ♀ Fundulus majalis ♂	Pœciliidæ Pœciliidæ	Haplomi Haplomi	1	78	Hatched.
Fundulus majalis ♀ Fundulus heteroclitus ♂	Pœciliidæ Pœciliidæ	Haplomi Haplomi	1	90	Up to hatching.
Fundulus heteroclitus ♀ Menidia notata ♂	Pœciliidæ Atherinidæ	Haplomi Acanthopteri	10	70–93	
Menidia notata ♀ Fundulus heteroclitus ♂	Atherinidæ Pœciliidæ	Acanthopteri Haplomi	4	14 to nearly 100	
Fundulus heteroclitus ♀ Menidia gracilis ♂	Pœciliidæ Atherinidæ	Haplomi Acanthopteri	3	About 100	
Fundulus heteroclitus ♀ Gasterosteus bispinosus ♂	Pœciliidæ Gasterosteidæ	Hoplomi Hemibranchii	3	18–86	
Gasterosteus bispinosus ♀ Fundulus heteroclitus ♂	Gasterosteidæ Pœciliidæ	Hemibranchii Haplomi	3	45–100	
Fundulus heteroclitus ♀ Apeltes quadracus ♂	Pœciliidæ Gasterosteidæ	Halpomi Hemibranchii			

Fundulus heteroclitus ♀ Eupomotis gibbosus ♂	Pœciliidæ Centrarchidæ	Haplomi Acanthopteri	6	Less than 1%
Fundulus heteroclitus ♀ Tautogolabrus adspersus ♂	Pœciliidæ Labridæ	Haplomi Acanthopteri	5	17–90
Tautogolabrus adspersus ♀ Fundulus heteroclitus ♂	Labridæ Pœciliidæ	Acanthopteri Haplomi	1	16%
Fundulus heteroclitus ♀ Tautoga onitis ♂	Pœciliidæ Labridæ	Haplomi Acanthopteri	3	40–66⅔
Fundulus heteroclitus ♀ Stenostomus chrysops ♂	Pœciliidæ Sparidæ	Haplomi Acanthopteri	4	30–70
Fundulus heteroclitus ♀ Cynoscion regalis ♂	Pœciliidæ Sciænidæ	Haplomi Acanthopteri	10 Many	20–92 17–92
Fundulus diaphanus ♀ Eupomotis gibbosus ♂	Pœciliidæ Centrarchidæ	Haplomi Acanthopteri	4	23–70
Gasterosteus bispinosus ♀ Apeltes quadracus ♂	Gasterosteidæ Gasterosteidæ	Hemibranchii Hemibranchii	1	17
Apeltes quadracus ♀ Gasterosteus bispinosus ♂	Gasterosteidæ Gasterosteidæ	Hemibranchii Hemibranchii	2	17½
Gasterosteus bispinosus ♀ Tautogolabrus adspersus ♂	Gasterosteidæ Labridæ	Hemibranchii Acanthopteri	2	74–100
Tautoga onitis ♀ Menidia notata ♂	Labridæ Atherinidæ	Acanthopteri Acanthobteri	1	60
Menidia notkta ♀ Tautoga onitis ♂	Atherinidæ Labridæ	Acanthopteri Acanthopteri	1	80 +
Gasterosteus bispinosus ♀ Tautoga onitis ♂	Gasterosteidæ Labridæ	Hemibranchii Acanthopteri	1	100

TABLE 9.—Continued.

CROSS.	FAMILY.	ORDER.	NUMBER OF CROSSES MADE.	PER CENT. OF IMPREGNATION.	REMARKS.
Gasterosteus bispinosus ♀ Menidia gracilis ♂	Gasterosteidæ Atherinidæ	Hemibranchii Acanthopteri	3	50-100	
Tautoga onitis ♀ Tautogolabrus adspersus ♂	Labridæ Labridæ	Acanthopteri Acanthopteri	2	95	
Tautogolabrus adspersus ♀ Tautoga onitis ♂	Labridæ Labridæ	Acanthopteri Acanthopteri	1	Large per cent.	
Gasterosteus bispinosus ♀ Menidia notata ♂	Gasterosteidæ Atherinidæ	Hemibranchii Acanthopteri	2	70-100	
Menidia notata ♀ Gasterosteus bispinosus ♂	Atherinidæ Gasterosteidæ	Acanthopteri Hemibranchii	1	100	
Opsanus tau ♀ Menidia notata ♂	Batrachoididæ Atherinidæ	Plectognathi Acanthopteri	1	8%	
Opsanus tau ♀ Menidia notata ♂	Batrachoididæ Atherinidæ	Plectognathi Acanthopteri	1	76	
Opsanus tau ♀ Tautogolabrus adspersus ♂	Batrachoididæ Labridæ	Plectognathi Acanthopteri	1	33⅓	
Coregonus clupeiformis ♀ Argyrosomus artedi ♂	Salmonidæ Salmonidæ	Isospondyli Isospondyli	2	Large per cent.	Hatched.
Argyrosomus artedi ♀ Coregonus clupeiformis ♂	Salmonidæ Salmonidæ	Isospondyli Isospondyli	2	Large per cent.	Hatched.
Cristivomer namaycush ♀ Savelinus fontinalis ♂	Salmonidæ Salmonidæ	Isospondyli Isospondyli	1	Large per cent.	Hatched and reared to fingerlings.

SUMMARY OF EXPERIMENTS.

INTRODUCTORY.

In the preceding detailed account of the various crosses effected are included combinations between forms of teleosts, ranging from closely related species within the same genus to species belonging to widely separated orders. Their relationships are summarized below, the figures set opposite each indicating the number of different combinations made in each group:[1]

Between different species of same genus	2
" " genera of same family	4
" " families of same order	1
" " orders of same class	17

A number of interesting facts appear from the above table and from a closer inspection of the more detailed Table 9. In all the crosses attempted with the exception of the cross in which Opsanus tau was used as the male, impregnation was possible. The sperm of the single Opsanus tau specimen used was not ripe in the three combinations attempted, so that it is impossible to say whether these crosses are possible.

CHARACTER OF IMPREGNATION.

In many of the crosses the impregnation was wholly normal. In some there was in addition to the normally impregnated eggs, a varying number of dispermic and polyspermic impregnations. Among the abnormally impregnated eggs the dispermic was very much more common than the polyspermic condition. In the dispermic eggs the protoplasmic disc, as is well known, falls at once into four cells. Sections of these conditions show that two male pronuclei fuse with the egg pronucleus; whether additional spermatozoa enter such eggs, but remain functionless so far as early cleavage is concerned, I am not able to say. In the polyspermic eggs the protoplasm falls at once into six or more cells. The cases coming under my observation in which many cells at once appeared, have been rather rare.

PERCENTAGE OF FERTILIZATION.

A striking fact is the large percentage of eggs impregnated. In fully two-thirds of the crosses this ran above 50 per cent., and in many of the combinations it ran above 75 per cent. A glance at Table 9 will show that this is not in any way correlated with the nearness of relationship.

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[1] This is represented in more detailed form in Table 9.

The low percentage of impregnation, on the other hand, must be regarded in most cases, I feel sure, as due to unfavorable conditions of the milt, and in some cases to the unripe condition of the eggs. Males that have passed the height of their breeding season, or which may have been less able to endure the conditions of confinement in aquaria usually show a reduced fertilizing power compared to perfectly fresh and ripe individual. The testes were in all the experiments cut out, so that it is quite probable that in many cases imperfect milt was used. I was, furthermore, not able to establish any constant difference in the percentage of impregnations in reciprocals. Allowing for the influence of the condition of the milt in determining the percentage of impregnation, in all cases where a fair trial was made in reciprocal crossing of two species it was approximately as high in one direction as the other. It is interesting to note here that Kammerer '07 using fresh water fishes, found, among the few forms he used, two crosses, Perca fluviatilis x Acerina schraetser and Lucioperca sandra x Perca fluviatilis, in which it was possible to impregnate when the first named in each case was the male, but not if female. It is also impossible to fertilize the eggs of Aspro zingel with the milt from the following nearly related forms: Perca fluviatilis, Lucioperca sandra and Acerina sp?, but was able to fertilize them with the milt from the distantly related form Cottus gobio. It would seem from these experiments that fresh water fishes lend themselves less generally to hybridization than the marine species.

Kammerer's statement that the eggs of Aspro zingel are fertile to the sperm of the distantly related form Cottus gobio when they were immune to the three nearly related forms above indicated, because Cottus had a similar habitat, and had with this also acquired the power to fertilize this species is, of course, a mere fancy. If he had tried to cross this form with other distantly related forms he would probably have found that they, too, would fertilize the eggs regardless of their habitat relationship.

DEVELOPMENT.

In my study of the development of these various hybrids I have not attempted to get a complete morphological picture, nor have I paid much attention to the inheritance aspect. I have regarded development rather from a physiological standpoint. The main points of interest, therefore, have been, first, how generally and within what limits can the sex-products of the various forms of teleosts be grafted upon each other, so to speak, and start development. Second, How far will development proceed in the various combinations, and in what respects are the processes normal and abnormal?

In every combination effected the earlier phases of cleavage are passed through in a perfectly normal manner. The same is true of the later stages of cleavage excepting the rate of development. This will be further considered below. From the late cleavage on, the history of the different hybrids becomes much more varied. In those hybrids resulting from species nearly related—belonging to the same genus or to closely allied genera—most of the embryos may complete their development to the point of hatching, or beyond. Even among these, however, a number variable but much greater than in normal embryos, may show abnormalities along the course of their development, such as occur more abundantly in the hybrids between more distantly related forms. Hybrids between species more distantly related than above indicated, so far as my experiments go, never complete their development to the point of hatching. The stage to which they will go depends again upon the nearness of their relationship. In the more successful of such distant crosses Fundulus-Menidia hybrids, many of the embryos may go far enough to form fairly well developed eyes, ear vesicles, tail, muscles, central nervous system, heart, color patern, fins, etc., but many of these structures in the later stages are variously abnormal. A large proportion of all the embryos, however, fail to reach such advanced stage. From these hybrids we have almost every condition to such as obtains in the hybrids between Fundulus heteroclitus x Tautogolabrus adspersus, where none of the embryos go much beyond the closure of the blastopore, and where it is not possible to speak of the formation of organs. The more characteristic and striking abnormalities appearing beyond the cleavage stage in these various hybrids may be briefly considered.

In the last stages of cleavage and during the earlier phases of germ ring formation it is usually not possible to distinguish the hybrids from the normals excepting in the stage of advancement. In some combinations, such as Fundulus heteroclitus x Tautogolabrus adspersus, etc., one can very commonly see the formation of a rather large clear area under the blastodisc which is filled with a clear fluid. I have followed such eggs and they do not bring their development to as advanced a stage as those eggs of the same lots that do not show this abnormality. They may form a very good embryonic ring and shield and may overlap the yolk for a third of the way and there die. In a few cases the vesicle was observed to be so large as to act as the yolk ball so that the protoplasm attempted to encompass it.

Embryonic shield might form and even lay down the axis of the embryo. These, like the above, soon died. In all hybrid eggs, but particularly those obtained from distantly related species, the period of gastrulation is one of great mortality. The embryos usually enter upon the germ ring and shield stage rather normally and simultaneously, but from this period to the closure of the blastopore the greatest variation in stages obtains. In some of the less successful crosses most of the eggs never succeed in properly closing the blastopore, but come to a standstill so far as this process is concerned at various stages, and continue the rudimentary formation of an embryo in the embryonic shield.

These aborted embryos may in some cases remain alive for days, developing pigment, a rudimentary heart, pericardial cavity, etc.

A very common deformity in the more successful embryos is the failure of the tail to bud out so that the embryos, very generally, are too short. A striking instance of this fact appeared in the hybrids between Savelinus fontinalis, female, and Cristivomor namaycush, male. This cross is quite successful, and the writer has succeeded in rearing 2,300 of them to fingerlings. Among this lot, a very large per cent. were deformed, and in every instance the deformity occurred in the region posterior to the anus. The portion anterior to the anus was normal in every way so far as proportions are concerned. The same is true of the caudal fin. But the region between this and the anus showed all degrees of shortening, the extremes appearing as if the caudal fin were directly set into the body of the fish. The anal fin was often wanting altogether, even in some that had the caudal peduncle otherwise normally developed.[1] This process of the elongation of the caudal end of the embryo seems evidently a difficult one, giving rise to the common abnormalities in this region. In those crosses where a portion of the embryos succeed in laying down the fundamental organs such as the eyes, ears, brain, heart, muscles, etc., promise well to carry their development to completion. In every instance, however, regardless of how normal the organogenetic processes may at first be, they show a very clearly defined abortive influence in a short time. This begins to show itself shortly after the time when the circulation is established in the normal embryo. This fails to develop properly in all these hybrids that fail to complete their de-

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[1] A detailed description of these hybrids are reviewed in a separate paper.

velopment. The heart usually differentiates and a pericardial cavity forms which commonly distends to enormous proportions. This has the effect of deforming the heart usually into a much elongated structure. The yolk and the embryo may in some instances differentiate blood vessels, but I have only in one instance observed either the heart or blood vessels handling any blood. The result of this is that the embryo which may up to this period be quite normal in its developmental processes, has its food restricted to what may be directly absorbed from the yolk through other agents than the blood. That the embryo does thus obtain some food is evident from the progressive reduction of the yolk and the increased size, and the long continued life of the embryo.

The eyes in rare cases may be quite normal. From this condition all degrees of abnormalities obtain. The eyes are commonly too small, located too far forward and too low down. Often an eye is developed only on one side. The eye may be rudimentary to the extent of being only a large black pigmented area in the region of the forebrain. A large proportion of the embryo develop no indication of an eye.

The ear may develop as a vesicle which in some cases shows otoliths. Commonly this vesicle becomes must distended, appearing as a prominent projection on either side. The ear less frequently appears than the eye.

The central nervous system may be laid down, the brain even showing some of the primary divisions in the more successful embryos.

The notocord is commonly present. The embryos may develop a varying number of somites, and quite commonly when these are present, some of the cells become contractile so that the whole embryo undergoes movements.

The fins rarely appear, but in some instances the pectoral fins may be much larger than in the normal fish.

If an embryo is laid down at all it rarely occurs that pigment does not develop, both on the yolk and in the body of the embryo. In some cases this may be quite heavily developed, showing accumulations of large and highly branched chromatophores. In the better developed embryos a simple pattern may develop showing varying degrees of bilateral symmetry.

The rate of development of the hybrid egg compared with that of the egg species, was noted in many instances. Comparative tables are given above in the detailed descriptions of the various hybrids.

The earlier cleavage stages in every case was that of the species from which the egg was taken. This is true whether the rate of cleavage from the sperm species is more rapid or slower than that of the egg species. Thus. reference to Table 3, where Fundulus heteroclitus was the egg species and Tautogolabrus adspersus was the male species, the rhythm of cleavage follows exactly that of Fundulus, although that of Tautogolabrus is very much faster. The reciprocal shown in Table 4 shows that the rate again is that of the egg species—Tautogolabrus. This is true all the way through, but attention is called to the hybrids with Opsanus tau, where the cleavage rhythm is relatively so extremely slow. (Page 373.) These facts are in accord with many observations made by others, especially Driesch ('98) on Echinoderms. Newman ('08, '10) obtained the same results in his Fundulus heteroclitus—Fundulus majalis hybrids. Fischel ('06), on the contrary, maintains that the influence of the sperm in some of the Echinoderm hybrids, makes itself felt even in the first cleavage. It is important to note, however. that such influence as he can detect is always to slow the development. This is what I find everywhere, as will appear further on, but I have not been able to detect it during the early cleavage stages. This slowing of the developmental processes is to be looked upon as pathological, a sort of incompatibility of the two germinal substances in such cases as it occurs. If it is permissible, as some authors do, to speak of the rhythm of cleavage as a character of the organism, then all my experiments most clearly show that the rate of earlier cleavage of the embryo is uninfluenced by the sperm, and may be regarded as wholly determined by the egg.

In later cleavage and all subsequent stages. the influence of the strange sperm becomes apparent in all the cases that I have carefully watched. It should be said here that hybrids between the nearly related species were not studied in this particular, but only those between the more distant forms. The influence of the strange sperm was in every case to retard development, usually to a marked degree. regardless of whether the developmental processes in the sperm species was much more rapid or slower than in the egg species. Thus Tautogolabrus adspersus takes only from twenty-four to thirty-six hours to hatch. while Fundulus heteroclitus takes from ten days to fourteen days, the hybrids, using Fundulus as the egg species, are slower in their development than Fundulus itself. The tendency, then, among fish hybrids obtained by combining distantly related species, is to develop slower after their earlier cleavage stages, than the egg species. It is, therefore, interesting to note Newman's result where he found a distinct acceleration in the later cleavage stages and subsequently in the hybrids between Fundulus majalis, female x Fundulus heteroclitus. male.

GENERAL CONSIDERATIONS AND THEORETICAL.

SELECTIVE FERTILIZATION.

In a general consideration of these experiments, perhaps the most striking fact that appears is the uniformity with which it is possible to cross-fertilize the various species of teleosts. The percentage of eggs fertilized is in practically all cases a high one—fifty per cent., and, in the majority of cases. seventy-five per cent. or over. When one reflects upon the reason for one's astonishment at this. he finds it in the fact that we have all, those of us who have given the matter any thought at all, allowed ourselves to grow into the belief that there is a sort of specific affinity or adaptation existing between an egg and the spermatozoön of the same species. This assumption may or may not be true. So far as the writer has been able to determine, there is extant no evidence that this is the case in the animal egg. A possible exception is to be found in the experiments of Dungern ('01). who finds that in the eggs of the starfish there is a substance which is poisonous toward the sperm of the sea urchin, but not vice versa. It is easy to see that under such conditions the spermatozoa of the starfish would be favored.

On the other hand. we have experiments by Buller on all the groups of Echinoderms which seem to show that there exists no specific affinity, chemical or otherwise. between the egg and its own spermatozoön.

The writer is elsewhere publishing a detailed account of his experiments on selective fertilization in fishes. It may be proper, however, to briefly call attention in this connection to a few of the results he obtained. First. The fact above stated. that among these fishes it is possible so uniformly to cross-fertilize the different species lends no support to the "specific adaptation" theory. Second. When a lot of Fundulus heteroclitus eggs are given a chance at a mixture of two sperm. one of which is their own and the other a strange species (Menidia. for instance), the eggs do not necessarily show any preference for their own sperm. In the case above mentioned. for instance, the great majority of the eggs prefer the Menidia sperm to their own. In other combinations the proportion is about equal. In still others the eggs may select more of their own sperm. The factor seems not to be the relationship of the sperm, but its vitality and fertilizing power. Third, Experiments with various egg extracts and the like on the behavior of spermatozoa give no evidence of any attraction of an egg for its own sperm or any toxic influence upon the strange sperm. It seems, therefore, that in the case of these teleosts there is no evidence of any specific adaptation of the egg for its own spermatozoön.

How can we account for these varying degrees of failure in development in these various hybrids? This question is as old as our knowledge of the common infertility of hybrids. Why should an animal or plant hybrid carry its development in a perfectly normal and healthy manner up to the final stage of sex product formation, and yet at this point so commonly fail? To this question we have up to the present time no definite answer whatsoever.

DEGREE OF DEVELOPMENT AND SYSTEMATIC RELATIONSHIP.

In following the development of the various hybrids hereunder discussed there appears one period in the development to which we might ascribe the failure of development, more than any other: this is the defective development of the circulatory system. Development in most crosses proceeds often in a relatively normal manner up to the period of the differentiation of the heart, blood vessels and the blood. In all the hybrids here considered that succeed in forming a circulatory system at all may begin to develop the heart more or less normally, so that it regularly and vigorously pulsates but fails to differentiate the blood and blood vessels. As a result the heart manipulates no normal blood and, as a consequence, the food absorption of the embryo must occur through other channels than the blood. Following this period the embryos invariably begin to lag behind, the organs fail to properly differentiate, resulting in the stunted, sickly-looking, starved hybrid. It would seem that if it were possible in some way to help the hybrids to properly complete this system, development might be carried much further, perhaps up to the point of hatching. But in the case of some hybrids none of the embryos form a heart and a varying percentage of all hybrids fail to develop the heart at all, even though the more successful ones complete development. Furthermore, it often happens that the circulatory system is apparently properly established and the development carried to the point of hatching, or even beyond, but they soon die. Thus while it is undoubtedly true that the establishment of the circulation is a vital stage in the proper progressive development of the embryo and is followed in normal embryos by a period of rapid growth, the question still remains, why does the circulatory system fail to develop properly? Why do we have so many embryos stop their development before the period of heart formation, and why do we have so commonly failures to emerge from egg, or die shortly after, when in the latter the circulation, at least to all appearances, has been normal? If we consider the experiments tabulated in Table 9 from the view point of the correlation between the degree of development and the relationship of the species combined, we see at once that only those species that belong to the same genus, or to very closely related genera, will produce hybrids that develop to the point of hatching. Even within this group a difference in this respect can be observed between species very closely related, and species more distantly related. Thus Fundulus heteroclitus combined in either direction with Fundulus diaphanus will produce a large proportion of free swimming embryos. These two species, although the former is a marine and the latter fresh water, are structurally very closely allied. Fundulus majalis is much less closely related to Fundulus heteroclitus, although belonging to the same genus. When the latter is taken as the female a large proportion of vigorous fry are obtained. The reciprocal has never yielded me embryos that would emerge from the egg, although, with the exception of the yolk bag, normal in appearance. Then the species used belong to separate genera the proportion of embryos that emerge normally is, as a rule, much smaller than in the preceding condition.

All species that are more removed from each other than closely related genera, fail to produce hybrid embryos that will complete development to the point of hatching. Among this latter group of hybrids the stage to which development is carried varies considerably in the different combinations. This, too, can be roughly correlated with the relationship of the species combined, so that two species belonging to distantly related orders like Fundulus heteroclitus x Tautogolabrus adspersus give rise to hybrids that can not go much beyond the closure of the blastopore, while if the same form is crossed with its nearer relative, Menidia notata, development proceeds very much further although stops far from the point of hatching. This will be further taken up below.

We produce, then, among fishes a series of hybrids that range in success from those in which none of the embryos develop very much beyond the "blastopore" stage though intergradations to those in which the embryos hatch normally and grow into adults, probably fertile creatures, and this series is correlated with the systematic relationship existing between the two species crossed.

The work of Guyer ('00) on the spermatogenesis of hybrid pigeons suggests that in the final formation of the sex products, difficulties arise in the synopsis of the male and female chromatim material, resulting in abnormal spermatozoa. Stated in more general terms in the final formation of the sex cell the developmental and hereditary substances from the two parents, fail to work harmoniously, giving rise to abnormal development. It is conceivable that an analogous process takes place in those hybrids that are arrested much earlier in their development. Indeed, the prevailing habit of thinking of developmental and hereditary determinants in terms of units of some sort, suggests at once to our minds some such picture as above indicated. In two nearly related species the developmental mechanisms are so nearly alike that no serious conflicts, so to speak, arise except possibly in the very last stages, namely, the formation of the sex cells. As a result, the development may be completed or all but completed. When, however, two distinctly related species are combined we have to do with two developmental mechanisms that are more divergent, and the conflict develops early in the life of the organism with the consequent modification of development, varying with the relationship. It is difficult to find any appearances in my hybrids that specifically support this view. It would seem that at least occasionally there would appear specific modifications to the influence of the sperm over the egg species. Thus it should be expected that the mode and rate of cleavage, the time and method of gastrulation, etc., should vary in a manner to be in a measure at least due to the specific characteristics of the developmental mechanisms of the sperm species. But this is just what one does not find. The whole process of hybrid development presents the picture of a pathological embryo, such as one sees when they are subjected to an unfavorable condition, such as foul water, insufficient oxygen, unnatural chemical media and the like. It is simply an arrest with subsequent gradual deterioration of the tissues. Thus the monocular condition is likely to result if the optic vesicles fail to form properly and the anterior brain-vesicle becoming pigmented in the cyclopian eye, or only one side develops the vesicle and becomes pigmented. The slender strangulated heart may be accounted for by the abnormally large pericardial cavity which develops, across which it becomes stretched. The large pericardial cavity may be the result of the abnormal method of yolk absorption due to the failure of the blood vessels to differentiate.

I have for three or four years looked upon these phenomena in my own hybrid experiments as a process akin to that which obtains in the transfusion of blood of strange species. The well known results of Landois ('75), Friedenthal ('99) and others bring out the important fact that the hæmolytic power of the bloods of two species varies in intensity with the nearness of relationship of the species. In general two very closely related animals will permit the transfusion of their bloods with no or relatively slight hæmolytic action. As the forms become divergent in relationship the toxic action becomes progressively greater. In a similar manner it has been shown that other tissues than blood act toxically. Among these are spermatozoa. The process in hybridization may be conceived something as follows: When the sperm brings its material into the strange egg in fertilization it brings with it the substance capable of poisoning the egg substance or vice versa. We may suppose that the toxic action does not manifest itself at once because of the relatively small proportion of the sperm substance compared to that of the egg. Consequently early cleavage stages are in all cases passed through in a normal manner. As, however, the nuclear material grows and becomes more generally distributed through the cytoplasmic mass as cleavage proceeds, the toxic action becomes manifest in the retardation of the cleavage and subsequent developmental processes. The intensity of the effect will vary with the degree of toxidity existing between the two species concerned. In the cases of fishes where cross fertilization is so generaluy possible it should be possible to get a measure of this in the faithfulness with which the embryo reproduces the normal developmental processes in the earlier stages, and the stage at which these become arrested.

In the transfusion of bloods we have seen that the toxidity varies rather closely with the systematic relationship of the animals. My experiments so far as they go, show that this same law holds in hybridization, and when taken in connection with what is already well known about the production of so-called "successful" hybrids, I think, may be interpreted as furnishing evidence for this view.

In order to obtain a somewhat more definite idea of the influence of a strange sperm upon the developmental processes, I have made a somewhat careful comparison of the final stages of a series of hybrids all of which had the same species, Fundulus heteroclitus, for the female but different species for the male, these latter varying in their nearness of blood relationship to the egg species. These males fall into four separate groups of two species each. The male species in each group are closely related, but the different groups vary in their relationship to the egg species from that of the same genus to that of most widely separated orders. These groups are as follows:

Group 1	Fundulus heteroclitus	×	Fundulus majalis.
	" "		" diaphanus.
Group 2	" "		Menidia notata.
	" "		Menidia gracilis.
Group 3	" "		Gasterosteus bispinosus.
	" "		Apeltes quadracus.
Group 4	" "		Tautogolabrus adspersus.
	" "		Tautoga onitis.

In group 1, Fundulus majalis and Fundulus diaphanus will hybridize and bring their development to hatching. The same is true of the two species of Menidia in group No. 2. In group No. 3 the two species of sticklebacks will cross and hatch, although I have been able to rear the embryos for only a very short time. The Cunner and Tautog of Group No. 4 will likewise cross and, although many abnormalites occur, some of the embryos will hatch in a normal manner. When, however, these forms are crossed with Fundulus heteroclitus very divergent results are obtained, although in every case most of the eggs are impregnated. In the first group the embryos largely hatch and may be reared. Among the normals may be found various abnormalities, but these are relatively rare. In the remaining groups the embryos never hatch, although in some cases may remain alive in the egg for three or four weeks. But each of these groups go to a characteristic stage of development and show characteristic abnormalities. In all of the last three groups the mortality is great during the period from the formation of the germ ring to the closure of the blastopore.

In group 2 a varying number may go far beyond this stage forming normally the early stages of the eye, ear, heart, notocord, somites, etc. Although the early stages in the formation of these organs may be normal, It soon becomes apparent that the further processes becomes aborted. The blood vessels do not properly differentiate, the pericardial cavity becomes very large and the heart is commonly drawn out to a filamentous form. This continues to beat until the death of the embryo, but does not handle any blood. The eyes do not attain their full size, and may be poorly pigmented. They often are abnormally set so that they occupy the forepart of the head. This may fuse into a median single eye or may be present on one side only. The ear vesicles often become large and inflated, giving rise to a large rounded prominence on each side. The pigment cells are very finely developed, show a tendency to a pattern and bilateral symmetry, but there is a lack of uniformity in this in the different embryos. The embryos are shortened and may develop abnormally large pectoral fins. It is not necessary to give more than a general description at this place.

Even within this group it is very easy to distinguish between the hybrids in which the Menidia notata is used as the male from those in which the Menidia gracilis is the male. The development of the former is more successful in those that pass the blastopore closure stage, although my experiments show that the mortality is greater at this point. The pigmentation is better developed and the various organs above mentioned are laid down much more normally. As a consequence fewer and less pronounced abnormalities occur. In the Fundulus-Menidia gracilis cross it is not uncommon to have only one eye formed. This may be lateral or may be median. The eyes are commonly set much further anterior so as to occupy the front of the head than in the nearly related cross.

In the crosses of group 3 we obtain quite a different series of hybrid embryos. None of these will develop as far along as those in group 2. There is the usual large mortality preceding and at the blastopore closure stage. The more successful embryos are much shorter, the pigmentation is much less perfect, the black usually predominates, the eyes are never normal, and often altogether wanting, and the life of the embryo is shorter. The heart and pericardial cavity is much the same as in the Menidia hybrids, although I have seen no attempt to develop vessels on the yolk. Their embryos show in every way that the developmental processes have deteriorated much earlier than in the Menidia crosses.

When we come to group 4 we have a still more pronounced abortion of the developmental processes. Many of the embryos close the blastopore after a fashion, but the embryo is always much shortened, usually being a mere streak of protoplasm. These embryos do not lengthen to form a tail, they form no eyes. Occasionally one or two poorly developed ear vesicles show; pigment is irregularly and rather sparingly developed on both the embryo and the yolk. These cells are practically all black with a few small, poorly developed brownish ones. The heart may develop into a protoplasmic pulsating mass showing no definite form. The pericardial cavity is poorly developed or wanting. These embryos may remain alive for a week or ten days, but never as long as the hybrids of the two preceding groups.

We can see from the foregoing that within the narrow limits of the species covered, that the more distantly two species are separated in their blood relationship when crossed, the earlier the developmental processes come to a standstill. The writer, of course, thoroughly appreciates that the foregoing facts are not necessarily evidence in favor of the view taken. He desires merely to emphasize the analogy existing between the conditions of hybridization and the known conditions of blood transfusion and the like. His belief that this analogy is a significant one has been strong enough to lead him into further, more direct experiments along this line. The writer may even be permitted to express a hope of his that it may be possible to control the processes of hybridization in a manner similar to that which has already been brought about in the field of immunity.

SUMMARY.

1. The eggs of any of the species of teleosts tried may be impregnated by the sperm of any other species tried.

2. The number of eggs fertilized is usually great, i. e., 75% or more. This bears no relation to the nearness of relationship of the two species concerned.

3. Normal impregnation is the rule, di- and polyspermy being the exception.

4. Development in its early stages proceeds normally, the deleterious effects of the two strange sex products upon each other showing only at later cleavage or subsequently.

5. The rate of development in the early cleavage stages is always that of the egg species. Any effect of the strange sperm upon the rate of development shows itself by slowing the process regardless of whether the rate of the sperm species is faster or slower than the egg species.

6. A period of great mortality in the developing hybrids is gastrula-

tion. If the heart is formed, although it pumps no blood, the embryo may remain alive for a considerable period, yolk absorption taking place to a varying degree. If the heart handles blood and bloodvessels are differentiated, the embryo is likely to develop to the point of hatching.

7. The numerous abnormalities appearing in the hybrid embryos are due to a deterioration in the developmental processes, resulting probably from the poisonous action of the sex products upon each other.

8. The success of the hybrids, i. e., the stage to which any given hybrid will develop, is correlated with the nearness of relationship of the two species used.

9. The mixing of unrelated sex products is looked upon as analogous to the transfusion of unrelated bloods, the more distantly related the two species concerned the greater their toxidity.

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