CHAPTER III

EXPERIMENTS IN CROSS-FERTILIZATION

A NUMBER of attempts have been made to fertilize the eggs of one species of frog with the spermatozoa of another species. Rusconi experimented in 1840 with the toad (♂) and the green water-frog, Rana esculenta (♀). Lataste in 1878 attempted to cross-fertilize the eggs of different species of urodeles with Pelobates fuscus and P. cultripes.

EXPERIMENTS OF PFLÜGER AND OF BORN

The most extensive and important work is that of Pflüger ('82) and of Born ('83). These investigators have made a large number of experiments in crossing different races and species of Anura. When the sperm of Rana fusca was placed with the eggs of Bufo vulgaris, the eggs segmented and developed as far as the "morula" stage, and then without exception died.[1] Conversely, when the sperm from B. vulgaris was used with the eggs of R. fusca, no result followed, not even the segmentation of the egg (except in one experiment where two eggs out of one hundred divided irregularly). Eggs of R. fusca placed in a water-extract of the testes of R. esculenta remained unfertilized. But eggs of R. esculenta placed with the sperm of R. fusca developed regularly, with few exceptions, as far as the blastula stage, and then died. Crossing various species of Tritons gave no results. But eggs of Rana fusca were acted upon by the sperm of Triton alpestris and Triton tæniatus, inasmuch as they began to show irregular cleavage-lines. Later they died. The reverse cross gave no result.

Rana fusca and Rana arvalis are very similar in appearance, but are apparently separate species. Cross-fertilization was

[1] Pflüger ('82).

here possible (R. fusca, ♂, R. arvalis, ♀). Tadpoles developed from the crossed eggs, and some of these ultimately transformed into frogs. Pflüger got similar results with the same species, and also found that the reverse cross (R. fusca, ♀, and R. arvalis, ♂) gave no result. Born found that the eggs of Bufo cinereus could readily be fertilized with the sperm of Bufo variabilis. All the eggs segmented regularly, the larvæ left the jelly, and developed into frogs.

In respect to the closeness of the relation between the species, Born says that we can be quite certain that the two species of Rana arvalis and R. fusca are much more nearly related than the two species of Bufo. The success of cross-fertilizing depends apparently less on the degree of relationship, as shown by the similarity of color and habits, than on the similarity of the male sexual products (Pflüger). Although R. fusca and R. arvalis seem to be very closely allied species, they have very different spermatozoa; in fact, the spermatozoa are as different as the spermatozoa of R. fusca and R. esculenta.[1] The two species of toads (Bufo) have very similar spermatozoa, which differ only in size, but this difference is so slight that, were the two kinds mixed together, one could scarcely distinguish between them. It is *apparently* owing to the difference in form of the spermatozoa of the R. fusca and R. arvalis, and to the similarity of the spermatozoa of B. cinereus and B. variabilis that the results are due.

Pflüger has made a large number of reciprocal crosses between different *races* of R. fusca. "The different races are as fertile *inter se* as are individuals of the same race." Pflüger concluded, after comparing the results of all of his experiments on cross-fertilization, that in general *those spermatozoa are most successful* for purposes of cross-fertilization *that have the thinnest and most pointed heads.* That in general those eggs are most easily fertilized that belong to species having spermatozoa with thick heads. The results, then, he thought, depend largely upon mechanical conditions; for where the head is small and pointed, the spermatozoön can bore its way more successfully into the eggs

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[1] R. arvalis and R. esculenta have similar sperm. Born and Pflüger found that the crossed eggs segmented irregularly, and that later the embryos all died.

of its own and of other species. If the head is large, the spermatozoön can force its way only into those eggs that are adapted to spermatozoa with large heads. For instance, the spermatozoa of R. fusca have thinner heads than any others, and the head is, moreover, very pointed. These spermatozoa can fertilize eggs of nearly all other species (R. arvalis, R. esculenta, B. communis). Conversely, the thick-headed spermatozoa of R. arvalis and the blunt-headed spermatozoa of R. esculenta cannot get into the eggs of R. fusca.

The spermatozoön of B. communis, which has a *very pointed* but *somewhat larger* head than that of R. fusca, appears nevertheless to be able at times to penetrate the eggs of R. fusca and to fertilize them. That the spermatozoön of Triton can enter the eggs of R. fusca is explained very easily when we remember that the sharp thin head of the Triton spermatozoön is best adapted of all species to penetrate any egg. We see, too, that the thick-headed spermatozoön with a blunt anterior end, such as those of R. arvalis and R. esculenta, cannot fertilize the eggs of any other species. And finally, to confirm the conclusion, we find that these two species, R. arvalis and R. esculenta, which have large-headed spermatozoa, are alone capable of *reciprocal* crossing. Pflüger believed that the eggs have the greatest capacity for cross-fertilization at the height of the breeding season, and the same statement holds, but in a much less degree, for the spermatozoa.

Experiments on Other Forms

Hertwig has objected to Pflüger's conclusions on the ground that the eggs of the sea-urchin are much more capable of cross-fertilization after they have begun to suffer change either from being kept some time in sea-water, or from the application of drugs. He thought that the frogs kept by Pflüger had been also under artificial conditions. Further, Hertwig concluded, from his results on sea-urchins, that the possibility of crossing does not depend entirely upon the external conditions, but to a large extent upon some unknown property of the egg. Eggs in good condition are able to prevent the entrance of foreign spermatozoa, but as soon as they begin to lose their irritability, they can no longer resist the entrance.

Born obtained some interesting results as to the relations existing between the number of spermatozoa in a fluid-extract of the testis and the power of the fluid-extract to fertilize eggs. He insists that in some cases there is a necessary connection between the two. It is far from clear how this is possible, and the result may depend on other causes which are introduced along with the solutions employed. Moreover, the further question of polyspermy of such eggs complicates the results. Born believes that many cases of irregular segmentation of crossed eggs are due to the entrance of several or many spermatozoa into the egg, which act as centres for protoplasmic accumulations. Such a segmentation he calls "barock" segmentation. On the other hand, Pflüger suggests that the *irregular* cleavage of certain of the crossed eggs is the result of the disintegration of the male pronuclei, so that the chromatin is scattered, and then acts on the protoplasm, producing an irregular division.

Recent results have shown that polyspermy is a normal occurrence in some amphibian eggs, and, despite the presence of several spermatozoa, normal cleavage and normal embryos result. The changes that take place within the cross-fertilized eggs must be more carefully studied before a final decision can be reached in regard to the meaning of some of the experiments described above.

We must not confuse two factors that enter into the problem of cross-fertilization. On the one hand, the spermatozoön may not be able to push through the gelatinous coatings of the egg, or it may not be able to bore through the outer surface of the egg itself, or it might be unable to enter the protoplasm if the latter were entirely free from its coats.[1] On the other hand, even if the spermatozoön could successfully enter and combine with the female pronucleus, it does not follow that the egg would develop. We now know that so many factors enter into the problem of fertilization of the egg that it is not surprising when we find that two pronuclei that have ever so slight differences are not able to carry out the complicated machinery of cell-division and development.

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[1] As in the case of naked pieces of protoplasm of the egg of species of sea-urchins.

The eggs of the starfish can be fertilized by the spermatozoa of the sea-urchin, — forms much more different than any two species, genera, or even families of frogs, and the early stages of segmentation, and the formation of a swimming blastula and gastrula may be passed through; but the later embryonic development is not carried out, and after a time the gastrulas die.[1]

Hertwig's experiments ('77) on polyspermy in the eggs of echinoderms show that when several spermatozoa enter the same egg a karyokinetic spindle is formed around each of the resulting male pronuclei and many or all of the pronuclei divide. Often the spindles are so near together that they mutually influence one another and most complicated karyokinetic figures result. Subsequently the protoplasm breaks up around the pronuclei in a most irregular way, and generally such eggs do not give rise to even the earliest stages of development. The phenomenon is so similar to the "barock" segmentation of the frog's egg that it seems possible that in the latter the result is brought about in the same way as in the echinoderms.

EXPERIMENTS OF RAUBER AND OF BOVERI

Rauber, in 1886, tried to carry out the following interesting experiment. The segmentation-nucleus of a frog's egg, one hour after fertilization, was removed by means of a fine pipette. The same process was carried out with a toad's egg. The nucleus of the toad's egg was then placed in the frog's egg that had had its nucleus removed, and the nucleus of the frog's egg was placed in the toad's egg. Unfortunately, neither egg developed. The results of such an experiment would be of the greatest importance if the experiment could be successfully carried out; for in this way we should hope to discover whether the characters of the embryo come from the nucleus or from the protoplasm of the egg.

Boveri, in 1889, made somewhat similar experiments with the egg of the sea-urchin. When the eggs are shaken in a small tube, they are broken into fragments, some with nuclei and others without. When a sufficiently large non-nucleated

[1] Morgan, '93, *Anat. Anzeiger.*

fragment is penetrated by one spermatozoön, the fragment develops. Such a fragment contains only half the number of chromosomes of the normal fertilized egg.[1] Boveri isolated some of these fragments, and said that they give rise to small embryos normal in structure. Boveri stated, further, that if a non-nucleated fragment of the egg of one species of sea-urchin is entered by one spermatozoön of another species, the resulting larva is like the larva of the father (*i.e.* it is like the larva of the individual from which the spermatozoön comes). If this result should prove true,[2] it would show that the nucleus and not the protoplasm determines the character of the larva.

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[1] Morgan, '95, *Anat. Anzeiger.*

[2] Seeliger ('95) and myself ('95) have repeated Boveri's experiment and have tried to show that the evidence on which Boveri based his conclusion in regard to the paternal character of the crossed larva is insufficient.