

THE CORRELATION BETWEEN CHROMOSOMES AND PARTICULAR CHARACTERS IN HY- BRID ECHINOID LARVÆ¹

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THE student of genetics who bases his researches on the development of echinoderms has become quite accustomed to the criticisms of his friends to the effect that his work can have little point until he can show them individuals of a second or a third generation, and he receives these criticisms at their full value.

Our ignorance on this subject has been clearly defined. First, little is known of the inheritance in the adult hybrids of the characters of the parent species, and second, it is not known that these hybrids become sexually mature.

The ready acceptance of these ideas has blinded us, in a measure, to the fact that for the solution of some of the problems of heredity, the echinoderms offer material of unusual advantage. The plants, insects and vertebrates are peculiarly adapted for the elucidation of the later aspects of heredity; the echinoderms are available for information concerning the processes which occur during and immediately after fertilization.

In the course of this paper I shall show that we are now in a position to predict certain characters in the adult hybrids from a knowledge of the germ cells of the ancestors and, granted the sexual maturity of the hybrid adults, to predict the character of their germ cells.

The evidence that I shall bring forward is based on a study of chromosomes. The advantages afforded by echinoderms for such a study, lie in the fact that not only may we study the chromosomes during the divisions of

¹ Read before the American Society of Naturalists, Princeton, 1911.

the normally fertilized egg, but we may study the chromosomes of the egg itself, *i. e.*, the maternal chromosomes, in artificially parthenogenetic eggs; we may study the chromosomes of the spermatozoan, *i. e.*, the paternal chromosomes, in fertilized enucleated egg fragments; and we may study the chromosomes in cross-fertilized eggs, identifying here those which have come from one parent or the other.

The first subject of which I shall speak is the correlation of certain chromosomes with sex.

The conclusions reached by Baltzer, from a study of *Echinus* and *Strongylocentrotus*, are known to most of those present. They find their readiest expression in the statement that "in Echinoids the female is digametic while the male is homogametic." I have expressed elsewhere the idea that this generalization is too broad and that the statement should be limited to the cases for which the condition was described; but I must do more than this. I can admit that apparent condition for but one of Baltzer's cases, that of *Echinus*, for his own illustrations for *Strongylocentrotus* indicate that this form is in agreement with another interpretation, and not with the one given.

In the forms which I have studied, the male is digametic, the female homogametic, as in the insects.

The first case that I shall submit is that of *Tripneustes esculentus* (*Hipponoë*), in which the evidence is especially clear, although not submitted to the ultimate analysis that has been given to the second case.

In *Hipponoë*, I have been able to show, by the study of cross-fertilized eggs, made in connection with straight fertilized *Toxopneustes* and *Hipponoë* eggs by two of my students, Miss Heffner and Miss Pinney, that a chromosome of peculiar form, namely hook-shaped, is transmitted by half of the *Hipponoë* spermatozoa, and that the eggs which receive it must become males.

The evidence here is clear. Such an element never occurs in straight-fertilized *Toxopneustes* eggs. It is

found in one-half of the straight fertilized *Hipponoë* eggs; it is found in half of the *Toxopneustes* eggs which have been fertilized by *Hipponoë* sperm, and it never occurs in *Hipponoë* eggs fertilized by *Toxopneustes* sperm.

There is then absolutely no appeal from the fact that in *Hipponoë* the spermatozoa are of two classes, one with, the other without, this idiochromosome.

The second case that I shall submit is that of *Lytechinus*, better known to us as *Toxopneustes*. Here I am able to give evidence gained by a study of straight fertilized eggs, artificially parthenogenetic eggs, fertilized enucleated egg fragments, and four crosses, the reciprocal *Hipponoë* \times *Toxopneustes* and *Arbacia* \times *Toxopneustes* crosses.

The study of straight fertilized *Toxopneustes* eggs showed two classes of zygotes, one with two V-shaped chromosomes, the other with three. This seemed in agreement with conditions in *Echinus*, although obviously there was no chance of tracing the source of the heterochromosome in such material.

Those who have devoted any attention to the chromosomes in Echinoids know the difficulties involved in such a study. The chromosomes are all small; most of them have the form of short, straight or slightly bent rods, while but three or four, at most, may be distinguished by peculiarity of form. Difficulties attend the study of even these chromosomes. The V's in *Toxopneustes*, for example, best seen in the anaphases of division, may have both arms brought into contact and so resemble a single, somewhat thickened rod.

Since this is true, it should be easier to determine size differences when only the haploid number is present than when the full number of chromosomes is in the division figure.

So far as I am aware, none of the cytological work on artificial parthenogenesis in Echinoids has been done with the idea of an individuality of form of chromosomes

in mind. The chief aim has been the determination of the presence of the haploid number of chromosomes and of the fact that auto-regulation does not occur. This is true for results published as late as 1910.

In my study of this material I have found that all of the eggs are alike, possessing among the others two V-shaped chromosomes.

In the fertilized enucleated egg fragments, two classes are evident. Half of the spindles show two V-shaped chromosomes in each anaphase plate while half show but one. I have no conclusive evidence for showing whether we are dealing with an X chromosome or with X and Y chromosomes. Counts are decisive for either view, and therefore valueless. I believe that this is a matter which can be decided only by a study of the spermatogenesis of these forms.

For some reason there is a belief in the tradition that in Echinoids the somatic number of chromosomes is thirty-six and in one case eighteen. Counts of perfect polar views of anaphase plates in *Toxopneustes* give the numbers thirty-seven and thirty-eight, but even from these I do not feel warranted in applying a sex formula.

It will be noted that the number of V's does not agree with our expectation from the straight-fertilized eggs. I can only say that my description is of the facts as I find them in my preparations. From this it would seem that the number of V-shaped chromosomes in straight-fertilized *Toxopneustes* eggs should be three and four. These facts are further substantiated by conditions in the *Hipponoë* \times *Toxopneustes* crosses.

The second point of which I wish to speak is that of correlation of chromosomes and larval characters. The only larval structure which has a form sufficiently definite to be used for comparison is the skeleton. The most advantageous forms for this purpose are those in which the pluteus of one parent has skeletal arm rods of a simple straight form and the other rods of the latticed type.

It is well known that an apparent confusion exists among observations on hybrid Echinoid larvæ, as to whether plutei of a maternal type, a paternal type or of mixed form are derived from certain crosses. Different results have been obtained by different investigators and by the same investigators working in different regions, or in the same region in different seasons.

From my own results I am convinced that there is no reason for believing that any of these observations are incorrect and that this dissimilarity of results may be traced to the influence of the constitution of the sea water at different times and places, to temperature, etc.

If we look into the matter carefully we find that there is a correlation between the fate of the maternal and paternal chromatin and the character of the plutei.

It is a remarkable fact that in the general literature of genetics, authors who have noted the fact of elimination of chromosomes, as shown by the researches of Baltzer and Herbst, have not also noted their retention in some cases, and the influences of this retained chromatin on the character of the larvæ.

It may be seen, by the examination of Baltzer's tabulation, given in his 1910 paper, that in the cases cited an elimination of presumably the introduced paternal chromosomes is followed by a pluteus of the maternal type, and that the retention of all the chromosomes may be followed in some cases by a pluteus of a mixed or intermediate type, in other cases by a pluteus of the maternal type. To these cases I must add the case of the retention of chromosomes followed by a pluteus of the paternal type, this being afforded by the *Hipponoë* ♂ \times *Toxopneustes* ♀ cross.

In this instance we have a clear example of *Hipponoë* dominance with respect to larval skeletal character, and this dominance is correlated with the retention of *Hipponoë* chromosomes.

I have not asserted that every *Toxopneustes* egg which is fertilized by *Hipponoë* sperm gives rise to a pluteus

with a *Hipponoë* skeleton. The largest percentage of the eggs does respond in this manner; a smaller percentage dies in the blastula and gastrula stage, and a still smaller percentage shows little or no trace of paternal influence.

If we take the eggs of such a fertilization and examine them during the segmentation stages, we shall see that in these eggs the greatest number show normal division figures, and by normal I mean those of the almost diagrammatic sort which may be seen in straight-fertilized *Toxopneustes* eggs, figures which give no indication of any chromosome elimination, while the smaller number show aberrant figures with varying degrees of elimination.

The normal division figures correspond in number to the plutei with the paternal type of skeleton and the aberrant figures to the plutei of intermediate and maternal character.

Clearly, then, in the hybrids of Echinoid crosses we may have dominance of one species or the other with respect to the skeleton, and this dominance may be transmitted by the egg or by the spermatozoan. Of the considerable number of crosses that I have made, only one, the *Hipponoë* ♂ \times *Toxopneustes* ♀ cross gives this evidence.

Some facts of interest are demonstrated by the *Arbacia* \times *Toxopneustes* crosses. These crosses are made with difficulty and I had never succeeded in getting them until the past summer. The chromosomes in *Arbacia* are much smaller than those in *Toxopneustes* and I had hoped that material from this cross would be of use when compared with my experimental *Toxopneustes* material. Here, however, I found that there is an elimination of chromosomes from the first, an elimination which may involve the rejection of not only the foreign chromosomes, but some of those of the egg as well, the result being that, in some instances, the full haploid number of neither is retained, and few zygotes pass through the blastula stage.

A further insight into the effect of the retention of foreign chromatin is given by partially arrhenokaryotic and partially thelykaryotic plutei. In such larvæ, with their asymmetrical bodies and skeletons, we may distinguish regional differences by nuclei of different sizes, and by a study of segmenting eggs we may find how these nuclei have arisen. In some instances we know that the fertilization processes have been so modified that subsequent divisions of the egg contain only paternal nuclear material and are paternal in character. In other regions part or all of the foreign chromatin is present and has modified development.

The conditions noted in the plutei find correlation in the following conditions in the egg.

1. Retention of all chromosomes and dominance of one species over another with respect to skeletal characters.
2. Elimination of part of the chromatin and dominance of one species over another with respect to skeletal characters.
3. Elimination of part of the chromatin and intermediate skeletal characters.
4. Elimination of part of both paternal and maternal chromatin and inhibition of development.

The first three, at least, may all occur in a given lot of eggs, and, since this is true, depend in part on chance, just as we may have imperfect mitotic figures in straight-fertilized eggs.

The second and third cases may indicate an incompatibility between the chromosomes and the cytoplasm of the egg; the fourth case, not this alone, but an incompatibility between the chromosomes themselves.

Finally as to our ability to predict the character of the adult from the characters of the larvæ.

It is clear that in *Hipponoë* and *Toxopneustes* we may predict sex, since we have seen that maleness and a heterochromosome of paternal origin are correlated. It is thus evident that if chemically fertilized *Hipponoë* and *Toxopneustes* eggs develop to sexual maturity these in-

dividuals will be females, the reverse of our belief concerning the naturally parthenogenetic egg of the bee. In cases similar to those described by Baltzer for *Echinus*, half the adults will be males and half females.

A prediction as to other characters is difficult to make, for the fact that one species is dominant over another with reference to larval skeletal characters give no idea as to other characters.

Nevertheless, in the cases of partial arrhenokaryosis which I have mentioned we should expect a region of the larval body containing only paternal nuclear material to place its stamp on that part of the adult body arising from it. A natural hybrid showing pure regional resemblances to one species or another is theoretically possible and would find its explanation in partial arrhenokaryosis.

For the inheritance of most characters we must fall back on our knowledge of natural echinoderm hybrids, which is increasing steadily.

We should expect that where parts of both contributions of chromatin have been retained and an intermediate pluteus has resulted the adult will be of the mixed type. One of the supposed Echinoid hybrids described by Mortensen fulfils this expectation.

The proof of these predictions lies in the rearing of hybrid larvæ to the adult condition. This we know to be possible. It requires only laboratory advantages of an unusual type, care, time and an accurate knowledge of the natural history of echinoids.