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CYTOPLASM AND HEREDITY.*

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It is too much to say that the days of controversy in biology are past. Yet most of us are now content to play the role of judge and jury, and abandon the less dignified role of advocate. I shall not, therefore, attempt to refute the arguments of my predecessors as a necessary preliminary to advancing evidence of exceptions. It may be granted that the work of Morgan, Bridges, Sturtevant, Muller and others, upon the fruit fly Drosophila, has demonstrated that differential factors of heredity lie in the chromosomes. Those who take comfort in the thought that these factors *may* lie in other bodies (perhaps cytoplasmic), which behave like chromosomes, but which can not be observed, and about which nothing is known, not even that they exist, may take that comfort without injury to any one but themselves. They are in the position of that famous student of heredity who, after a lifetime of biometric work, naively remarked that he saw nothing in the Mendelian work of the present century which refuted a single jot or tittle of his conclusions, but who did not think it worth while to admit that not a few of those conclusions, while still true, had, in the light of the newer work, become practically useless. They are putting new wine in old bottles.

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It may be granted that the cytological work of the past fifteen years has established an undoubted connection between chromosomes and sex. One may even magnanimously neglect to point out that in some cases, for example, in the phylloxerans and probably the rotifers, the sex-determining event, as shown by differences in the size of the eggs, precedes the differential behavior of the chromosomes, and that the chromosomes are therefore not players, but pawns. It may be admitted that the experimental work of Baltzer, Gates, Lutz, Stomps, and others, has fixed upon the chromosomes the responsibility of producing certain hereditary features of the organisms they studied.

Yet, after making all these admissions, it is possible to accept as demonstrated certain facts which plainly indicate an influence upon hereditary processes, of something else than chromosomes. It is my purpose first to point out a few of these facts; and second to show how we may cherish this evidence, without spewing the chromosomes out of our mouths, like the angel of Laodicea, and likewise without straddling.

Among the foremost evidence of the importance of cytoplasm in heredity is that derived from cases of inheritance only through the mother. Inheritance only through the mother is in strong contrast to one of the earliest evidences in favor of the nucleus as the bearer of hereditary factors. It was long ago pointed out that father and mother shared equally in fixing the nature of the offspring; but that the spermatozoa carried little or no cytoplasm, while the egg was, from the standpoint of volume, *chiefly* cytoplasm. The chief difference between egg and sperm is that the former is lumbered down with a mass of passive cytoplasm and yolk, from which the sperm is practically free. When, then, we find a case of inheritance only through the mother, there is left little room for any conclusion but that this inheritance depends upon the cytoplasm of the egg, or upon something included in the cytoplasm.

The facts in one such case are these. In the old-fashioned four o'clocks of grandmother's garden, *Mirabilis Jalapa*, there is a variety named *albomaculata*, which has variegated leaves. The structural basis of the variegation is the fact that the chromatophores in the yellowish white patches are not bright green, but more or less blanched. The amount of green and white varies greatly in different plants. Furthermore, whole branches may be green, other whole branches white.

Flowers borne upon green branches, if self-fertilized, give seed that produces only green offspring. Flowers upon white branches if self-fertilized, give seed that produces only white offspring, which soon die because unable to carry on photosynthesis. Flowers on variegated branches yield offspring some of which are white, some green, some variegated.

Crosses between flowers upon green branches and flowers upon white branches yield the important result that only the mother determines the chlorophyll character of the next generation. Correns found that when, in such crosses, the flower used as a female was on a green branch, the offspring were all green. If the flower used as a female was on a white branch, the offspring were all white. A flower on a variegated branch yielded seeds that produced variegated plants, regardless of whether the pollen came from a green, a white, or a variegated branch. As regards this color character, the offspring are always like the mother. Even in subsequent generations, there is no reappearance of the paternal character. Domination by the female is even more rigid in these garden plants than in human families.

What causes this peculiar course of heredity may be questionable; but Correns suggests that it is due to a disease transmitted only through the cytoplasm of the egg. No generalization can be made in regard to variegation, for in other plants this character is found to be inherited through the sperm also; but there appears to be no doubt that in Mirabilis it is a cytoplasmic character.

Similar evidence of cytoplasmic influence in heredity is to be found in what are called matrocline hybrids. When two crosses are made between two races or varieties, the mother coming from race A in one case, from race B in the other, these crosses are called reciprocal crosses; and the first generation hybrids from these crosses are known as reciprocal hybrids. In ordinary Mendelian cases, the two reciprocal hybrids are theoretically equal. If the chromosomes are the bearers of hereditary factors, and if there is no disturbance in the normal chromosome behavior, the reciprocal hybrids *should* be equal. But in certain cases they are not equal, each reciprocal being more like the mother which produced it. Unequal reciprocal hybrids which resemble the mother more than the father are described as matrocline hybrids.

The earliest cases of matrocline hybrids appear to be those between different orders or genera of echinoderms. These were not always reciprocal crosses. Such crosses have been made possible largely by the work of Loeb and others on the induction of artificial parthenogenesis, through chemical changes in the sea water. Loeb himself fertilized sea-urchin eggs with the sperm of starfishes and ophiurans. The larvæ were purely of the maternal (sea-urchin) type. Godlewski fertilized sea-urchin eggs with crinoid sperm. The larvæ were again purely maternal. The most obvious explanation in each case is that the type of larval development is determined by the cytoplasm of the egg. Too much stress is not to be laid upon this evidence, however, for recent work of Baltzer has shown that there may be irregularity of the behavior of the chromosomes in the two reciprocal hybrids; so that these matrocline hybrids may some day become most excellent evidence of the importance of chromosomes in heredity. This objection can hardly be urged against other experiments of Godlewski, in which fragments of sea-urchin eggs that contained no nuclei were fertilized with crinoid sperm. Even if irregularities in the behavior of the chromosomes occurred, and some of the chromosomes were lost, whatever chromosomes remained must have been paternal. Yet larvæ from these egg fragments were purely maternal in type. This result is not the universal one, it is true, for Boveri obtained precisely the reverse effect in another cross. But for those cases in which the larva produced by merogony (that is, the fertilization of egg fragments) is maternal, there seems little room for any other conclusion than that the cytoplasm of the egg is responsible.

Not all matrocline hybrids, be it pointed out in leaving this type of evidence, are evidence of cytoplasmic influence. There are matrocline hybrids in the evening primrose, Oenothera. But there are also patrocline hybrids in the same genus, that is, reciprocal hybrids that resemble the father more than the mother. Oenothera is probably not a lawless being, but so far its laws have baffled all its students. When patrocline hybrids are finally explained in Oenothera, the explanation may well be such as will also explain matrocline hybrids without an appeal to the cytoplasm. But this anticipated defection of the evening primrose from the ranks of matrocline hybrids which owe their maternal resemblance to the cytoplasm of the egg, will not weaken the evidence which other undoubted cases of cytoplasmic influence afford.

Further arguments against the chromosomes as holders of patent rights in heredity is found in the polarity of eggs. All eggs have differentiated regions, even when these are not visibly different. In most cases the animal pole becomes the aboral pole of the later gastrula, while the vegetative pole becomes the interior of the digestive tract. This polarity can be traced back, in some cases, into the early oogonial stages, and it is not improbable that it is continuous from one generation to the next. It is scarcely conceivable that this polarity is due to anything else than the cytoplasm.

Symmetry in many animals is likewise apparently independent of the chromosomes. This is particularly true of the insects and the cephalopods. In the back swimmer Notonecta, the last part of the egg to emerge in oviposition always forms the same part of the larva, and there is a bilaterality of the body that corresponds to a bilaterality of the egg. Since in the development of the insect egg the nucleus divides repeatedly before the daughter nuclei are shut off in separate cells, it is scarcely conceivable that a selective distribution of chromatin can occur with such minute regularity as to account for the regular location of the organs. Perhaps the mere shape of the egg, acting mechanically, may produce this symmetry; but in any case, it is not the chromosomes.

And finally, the case of the ascidian egg is important. These eggs contain various localized metaplasmic substances which can be traced into the muscles, the notochord, and the nervous system of the larva. If that part of the egg which contains one of these substances be removed before development begins, the corresponding part of the larva is missing. The cytoplasmic inclusions may not be organ-forming substances, but in that case the cytoplasm itself must exert a determinative influence.

The foregoing facts indicate a probable, in some cases almost necessary, influence of cytoplasm in heredity. They are not intended, however, to disprove the chromosomes hypothesis. It seems to me possible to hold the view that both chromosomes and cytoplasm have their influence; but they play different roles. Let us examine anew the facts we have cited to show the hereditary influence of cytoplasm. There was one case (variegation in Mirabilis) which may be explained as due to a disease transmitted by the cytoplasm of the egg alone. If the disease is due to an infection to the germ of which chromatin has so far proven immune, the transmission of variegation is no more heredity, it seems to me, than is intrauterine transmission of syphilis. But if by disease we mean merely a defect, then this defect is found only in the cytoplasm. Perhaps the results can be explained by assuming that the defect lies in the chromatophores themselves, that these are autonomous bodies arising only from other bodies like themselves, and that they are handed on to new generations only in the cytoplasm of the egg. This is clearly not opposed to inheritance through the chromosomes as a general phenomenon.

All the other phenomena listed above in support of cytoplasm as an agent in heredity involve only developmental stages. Polarity directly traceable to polarity of the egg has reference only to an early larval stage. The symmetry referred to differences of the cytoplasm, applies only to the embryo. The ascidians from which muscles or notochord were missing, due to removal of part of the egg, were observed only in larval stages. The matrocline hybrids among echinoderms have been observed only as larvæ. Unfortunately, it has been found impracticable to rear them to the adult stage. No one knows whether the reciprocal hybrids would or would not be dissimilar as adults.

In view of the fact that much of the evidence that the cytoplasm influences heredity comes from embryonic stages, may we not harmonize the once conflicting views regarding chromosomes and cytoplasm in the following manner? Barring such characters as variegation in Mirabilis, for which there is a special explanation, it may be assumed that the cytoplasm often (perhaps usually) determines the type of cleavage, the early course of development, and in large measure the larval characters, while the adult characteristics are determined by the chromosomes. With the developmental stages the student of heredity using the usual breeding methods has little to do. He may be pardoned a bias in favor of the chromosomes because he rarely studies larval characters. To the physiologist and morphologist, on the other hand, the rigid conviction of the geneticist that the chromosomes contain all the tools of his trade has not unnaturally been viewed with skepticism.

I claim no originality for the above attempt at harmony. The idea I have expressed was first propounded, I believe, by Conklin in 1908. I am, however, able to advance in favor of Conklin's view evidence which was not available when Conklin wrote. In my own work on rotifers I have discovered a case of matrocline hybrids which, unlike those of the echinoderms, were easily reared to the adult stage, at which time they were wholly alike. The facts of this case are as follows: The rotifers are one of the groups of animals that lay both parthenogenetic and sexual eggs. The former hatch regularly in 14 to 18 hours after laying, the latter remain in the egg a week or longer. Moreover, whereas all the parthenogenetic eggs usually hatch, only a fraction of the young developed in sexual eggs ever emerge. The proportion of sexual eggs hatching varies greatly in different lines. In line A, in the experiments above referred to, about fifty per cent of the sexual eggs hatched. They began to hatch about a week after they were laid, and two weeks later practically all had hatched that would hatch at all. The time spent in the egg was thus fairly uniform. Line B was strongly contrasted with line A both in the total number of sexual eggs that hatched and in the length of time spent in the egg. Only five per cent of the eggs of line B ever hatched. Morover, their hatching was spread irregularly over a period of five or six weeks.

The reciprocal hybrids obtained from crosses between these two lines were very unequal. When line A furnished the mother, line B the father, the eggs laid by the females hatched in one to three weeks, like line A, and about fifty per cent of them hatched, also like line A. When line B furnished the mother, the hatching of the eggs occupied four or five weeks, and the total number hatching was about thirty per cent. In both respects the hybrid eggs in this cross were intermediate between the parent eggs.

Here the reciprocal hybrids are very unlike, each being much nearer the maternal condition. But when new lines were obtained from these hybrid eggs, and these lines produced sexual eggs of their own, the two reciprocal hybrid *lines* were fully equal. Doubtless the inequality of the reciprocal hybrid eggs was due to the cytoplasm furnished by the mother; but when adults were developed from these eggs, and new cytoplasm was produced under the influence of the paternal as well as the maternal chromosomes, the eggs containing this cytoplasm were alike in the length and uniformity of the time of development, and in the total number hatching.

Whether the matrocline hybrids of echinoderms, so far observed only as larvæ, would on becoming adults show any less the characters of the mother than in the developmental stages, can only be conjectured. But the demonstration of such a change between embryonic and adult life in the rotifers supports Conklin's suggestion that it is the larval characters which, in animals in general, are influenced by the egg cytoplasm. When, however, in writing of the function of the chromosomes, Conklin states that they have only to do with the *details* of adult structure, I am unable to follow him. Nor, it seems to me, are some of the larval features which are conditioned by the cytoplasm, for example, the form of the larval skeleton of echinoderms, to be regarded as anything else than details. Geneticists, it is true, usually deal only with details; but that is because no mutations which involve radical changes in fundamental processes, and still leave the organisms capable of breeding with the parent form, have occurred. It seems much more probable that even the fundamental features of the adult are products of chromosome determination.

Cytoplasmic influence in heredity may appeal more to a certain type of mind if there is a mechanism through which it operates. That type of mind (or some other) has already found the mechanism. Hereditary importance is attributed by some to those bodies, found in the cytoplasm of many cells, called chondriosomes. One of the leading cytologists of America (who be it said is an ardent advocate of the chromosome hypothesis) admits that "probably" the chondriosomes have something to do with heredity. But I suspect that his admission was made chiefly to clear his conscience of any bias in the opposite direction. The only evidence that chondriosomes play any role in heredity seems to be that the cytoplasm plays such a role—and the chondriosomes are in the cytoplasm.

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