A COMPARISON OF THE CHROMOSOMES OF THE RAT AND MOUSE WITH REFERENCE TO THE QUESTION OF CHROMOSOME HOMOLOGY IN MAMMALS[1]

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The facts presented below owe their general interest to certain theortical considerations which have formed in part the background for a series of mammalian chromosome studies to which the writer has devoted his time for the past seven years. The question with which we are immediately concerned is this: May the chromosomes of the eutherian mammals be considered (in general) as having the same genetic make-up; for example, does the largest chromosome pair of different species carry the same genes or modifications of the same original genes? At first sight this would appear as a highly speculative problem for the cytologist to consider for, obviously, the final answer to this question lies with the geneticists of the future, but if we go on the assumption that the chromosomes represent the material substratum of genetic characters and hence in a sense that they are aggregations of genes, then it is possible for the cytologist to indicate in a provisional way the probable final answer.

The chromosome constitution of animals considered either from the standpoint of number or of size varies within wide limits; nevertheless, it has been a common experience of cytologists working in restricted fields to find that closely related species show a close similarity in their chromosome make-up. There is an easily recognized type number and deviations from this can be explained, usually, on the basis of an end-to-end fusion or to a breaking up (transverse fragmentation) of one or more chromosome pairs. The most striking example of this is found in the Acrididae which McClung and his students have studied so extensively, but animal cytology abounds with illustrations of this same condition as an examination of chromosome tables will convince any one (see HARVEY 1920, or WILSON 1925, p. 855).

The reason why chromosomes of related species are similar is undoubtedly phylogenetic, that is, related species have similar chromosome complexes because they inherited these from a common ancestor. Recent

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works in genetics and cytology have revealed unsuspected factors which would tend to stabilize this established chromosome constitution and to hold it at a constant level both quantitatively and qualitatively. The localization of sex determining factors in sex chromosomes in animals forms a very effective bar to a change in the chromosomes due to polyploidy as both the writer (PAINTER 1925a, 1925b) and MULLER (1925) have pointed out. There are various conceivable ways in which triploid or even tetraploid individuals could (and probably do) arise in nature, but such individuals could scarcely be perpetuated under natural conditions due to the complications associated with the sex chromosomes.

The loss of a chromosome pair, a whole chromosome or even a part of one chromosome due to what has been termed "genic balance" seems to be barred by the fact that such a loss is accompanied either by low fertility, sterility or low viability. Thus the absence of one of the very small fourth chromosomes in *Drosophila melanogaster* results in individuals of low fertility and viability and nullo-IV flies die. More recently GATES (1927), and the writer (1927) have shown that when only a part of one of the small chromosomes of the mouse is absent, (a normal mate being present) such individuals are extremely frail, and Gates has never been able to produce mice in which both chromosomes were affected presumably because of low viability. In this instance the chromosome involved is one of the smaller elements in a complex of forty chromosomes and represents a very small fraction of the whole chromatin mass. It thus appears that the upsetting of the normal genic balance which accompanies the loss of chromatin would probably bar the further perpetuation of such an aberrant chromosome constitution.

Just what effect the reduplication of a whole chromosome would have upon the individual is not clear (for animals) but the unstable character of the trisomic condition would require very special conditions for its perpetuation in the stable tetrasomic form, requirements which would scarcely obtain in nature.

Since the chromosomes represent directly or indirectly the physical basis of the genes, it follows that these same factors which have operated to keep the chromatin at a fixed level have also operated to stabilize the total number of gene loci and we should expect that nearly related animals having similar chromosome complexes would also show a similarity in the linkage relations of the genes. The work of METZ (1923) on Diptera, the only animal group extensively studied in this regard, seems to justify this expectation.

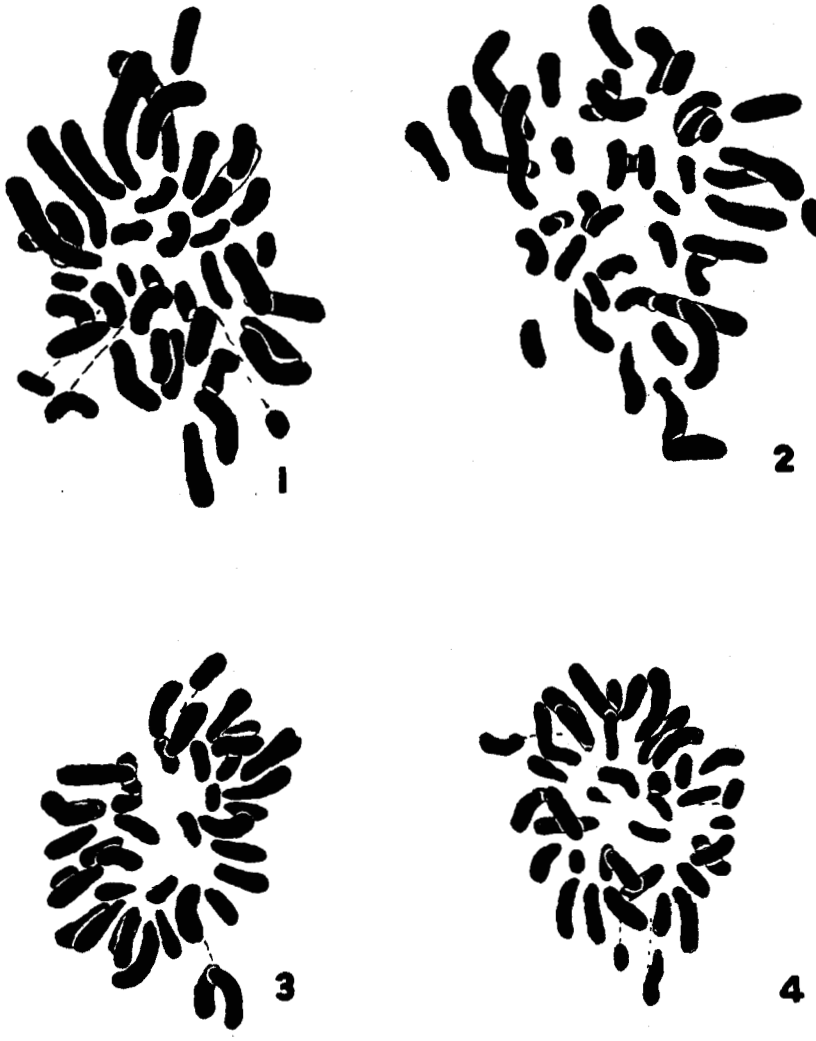
When the writer first began to study the chromosomes of the eutherian

mammals it was anticipated that a great variation in chromosome size and number would be encountered, but as work went forward and species from seven orders were studied it became apparent that the eutheria were much like a genus of insects in this regard. The average and the typical number for the group appears to be about 48, and deviations from this number are accompanied either by an increase in the proportion of small chromosomes, giving a higher number, or by a decrease in the proportion of small chromosomes resulting in a lower count (PAINTER 1925b). These observations accord well with the current conception that deviations from the type number in a small group can be explained on the basis of an end-to-end fusion of two or more pairs, or to a breaking in two of one or more pairs of chromosomes. If these are the only types of changes which have taken place in the eutherian orders and species then there should be an essential homology between the chromosomes of different orders and species and, what is of more interest to geneticists, a similarity in the arrangement of genes except where a break or a fusion has altered linkage values.

On the other hand, as I pointed out in a recent paper (PAINTER 1926), it is possible that, while the chromsome number has been kept at a high level in the eutheria due, perhaps, to some structural peculiarity of the eutherian cells, there may have been a great deal of shifting of chromatin within a chromsome or between non-homologous chromosomes which would greatly affect the arrangement of the genes without altering them in a quantitative way. If this shifting took place within a chromosome such as the inversion of a segment which STURTEVANT and PLUNKETT (1926) have described in *Drosophila simulans* as compared to *D. melanogaster*, it would require careful genetic tests to detect it, but if there has been a considerable shifting of chromatin between non-homologous chromosomes in related species a careful cytological study would reveal it. The extensive study of the chromosomes of rodents which has been under way at this laboratory for several years was undertaken partly with the view of gaining light upon this question.

The white rat (*Mus norvegicus*) and the common house mouse (*Mus musculus*) are placed by systematists in the same genus (*Mus*) and in view of the considerations and observations recorded above, it was anticipated that their chromosome complexes would be very similar. On the contrary, however, the complexes are very different, a fact which forces us to modify the point of view expressed in earlier mammalian chromosome studies.

The material on which this work is based consists of the testes of rats and mice preserved by Allen's modification of Bouin's fluid and subsequently treated in just the same way. In makings drawings much time has been devoted to the careful measurement of the chromosomes, using

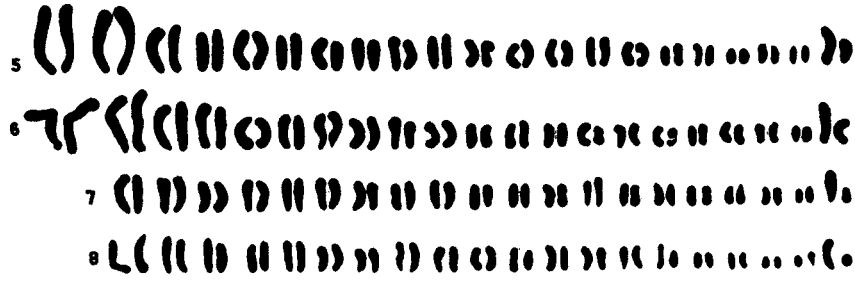


FIGURES.—1-4

the reflected image of the camera lucida and a pair of calipers, so that the finished drawing given below are as accurate as it has been possible to make them.

Mus norvegicus possesses 42 chromosomes and typical spermatogonial

chromosomes are shown in figures 1 and 2. These have been measured, copied and placed in serial alinement in figures 5 and 6, due consideration being given in the matching to any foreshortening observed by the microscope. The serial alinements of the elements of figures 1 and 2 (figures 5 and 6 respectively) shows that the rat possesses two pairs of very large



FIGURES.—5–8

chromosomes which are almost double the size of the third largest pair. From the third largest pair downwards the elements form a graded series. The X sex chromosome, as in other eutheria, is a medium sized chromosome but the Y is much larger than in forms heretofore studied. (The evidence for this is based on primary spermatocyte observations). The ratio of size between the smallest and largest elements (computed volume) averages about 1:8.

Mus musculus possesses 40 chromosomes and typical spermatogonial chromosomes are shown in figures 3 and 4. When these are placed in serial alinement (figures 7and 8) the chromosomes form a closely graded series, no element or pair being outstanding in size. The ratio of size between the smallest and largest (computed volume) averages about 1:5.

An attempt to express graphically the differences of the chromosomes of the two species is given in figure 9. The volume of each chromosome has been computed by measuring the length and average thickness as shown by my drawings (volume = length $\times$ thickness^2) and plotting the results in the form of a curve. Curve A (rat) and curve B (mouse)represent averages for the two alinements given for each species. In the rat (A) there is a sharp break in the curve between the second and third chromosome pairs, but from this point downward the seriation is more gradual. In the mouse (B), on the other hand, the seriation of volumes is so gradual that the curve approaches a straight line.

When one compares curves A and B and notes that except for the two large chromosome pairs of the rat, the curves are not unlike, the thought

will suggest itself that either the rat has gained most of the extra chromatin represented by the two largest pairs, or that the mouse has lost this material. It must be remembered, however, that while the unit of measure is the same in both cases (cubic mm for my drawings) that actually the rat spermatogonial cells represented are much greater in size than the

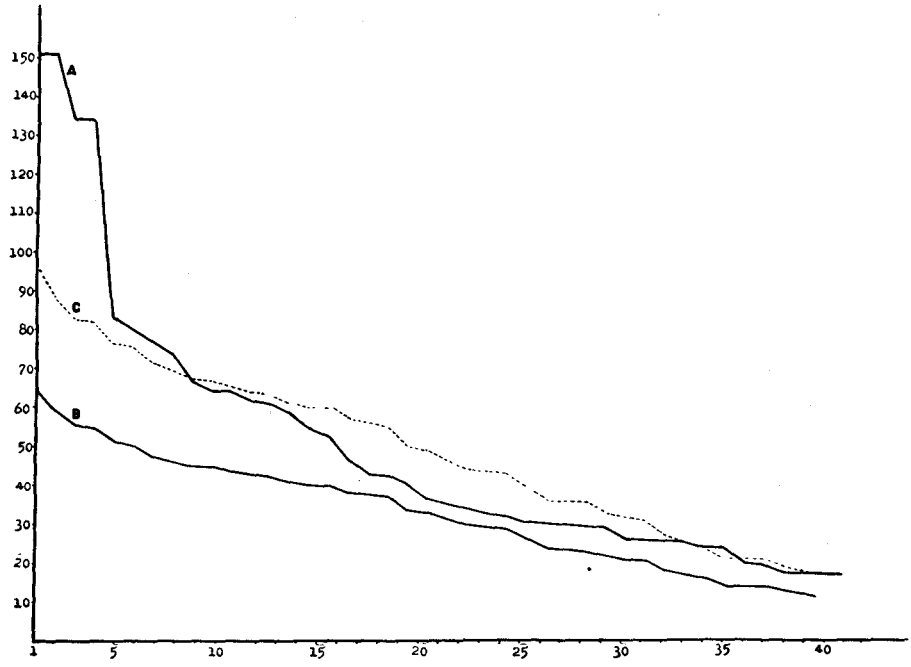


FIGURE 9.—The curves represent the volume of the chromosomes of the rat and mouse, as represented in my drawings, and measured in cubic millimeters. A is *Mus norvegicus*, B, *Mus musculus* and C, is *Mus musculus* with a correction made for difference in cell size between these two species

mouse cells, and since the size of a chromosome varies with the volume of the cell (within limits), we must correct for this difference in volume before we can legitimately draw any conclusions about gain or loss of chromatin.

To gain some idea of the relative volumes of the cells which we are comparing the large and small diameters in each case were measured, averaged and then the volumes computed assuming that the cells were spheres. The ratio of the volumes of the mouse and rat cells illustrated is 28:41. If the volume of the chromosomes, in a given tissue, is proportional to the volume of the cell, we should have to increase the volume of

the mouse chromosomes by about a half in each case to compare them directly with the rat chromosomes. This has been done in C of figure 9. Here we note that while the two large rat chromosome pairs are still far greater in volume than any of the mouse elements, that the volumes of the average sized chromosomes of the mouse are greater than in the rat. The total volume of the rat chromosomes as measured in A is 2130 cubic millimeters. The total volume of the mouse chromosomes (B) is 1327 cubic millimeters. The total volume of the mouse chromosomes as represented by C, is 1990 cubic millimeters. The difference in volume of chromatin in the rat and the mouse, when the difference in cell size is taken into account, is only about 140 cubic millimeters in favor of the rat in spite of the two pairs of large chromosomes and the higher chromosome number.

It would seem, therefore, on the basis of the measurements and calculations given above that the total volume of chromatin in the rat and in the mouse (2130 versus 1990 cubic mm for my drawings) is not very different, when differences of cell size are taken into account, and that we have no good ground for assuming that there has been any extensive loss or gain of chromatin in either species. This conclusion is to be expected, perhaps, in view of the known factors discussed in the first part of this paper which would tend to stabilize the total chromatin content in cells of related animals.

The cells selected for illustrating the chromosomes of the common rat and mouse are typical of what has been found during the past two years at this laboratory. A very large number of cells both somatic and germinal of the two species have come under observation and exact study, and the size relations illustrated have been consistently observed. The method of preservation and the subsequent treatment of the tissues has been the same in both cases. It is conceivable that rat protoplasm and chromatin might react somewhat differently from these structures in the mouse, but the reaction should be uniform, that is, to cause all the chromosomes to swell or shrink proportionally. The differences noted in the alinements of the two species can not be explained on the basis of technique.

The fact that the rat carries the higher number as well as the largest chromosomes precludes the possibility that the observed differences of the two species can be explained on the basis of an end-to-end fusion of two pairs of chromosomes (to give the mouse number) or if the mouse represents the original condition the breaking in two of one pair to give the rat number.

If there has been no great loss or gain of chromatin since the rat and mouse took their origin from a common stem, it is clear that at the present time there is a very different distribution of this material among the chromosomes of the two species. A simple shifting of material between two or three pairs of chromosomes is not adequate to make the alinements similar. If we wish to make the alinement of the rat chromosomes as represented in A of figure 9 similar to that of the mouse (C of figure 9) it would be necessary to remove a large amount of chromatin from the two largest pairs at least and to distribute this to a considerable number of medium sized rat chromosomes, a change which would involve a majority of the rat chromosomes. Or if we wish to make the mouse curve C correspond to the rat alinement, we should have to remove material from a number of medium sized mouse chromosomes and add this to the largest chromosomes. In either case the alterations necessary would be extensive and involve the majority of the chromosomes. We do not know, of course, which of the two species represents more nearly the original stem complex, indeed, the extensive differences which have been noted would suggest that both species have undergone considerable and independent changes in chromatin distribution since they originated.

The fusion of chromosome pairs and the translocation of chromatin have both been reported a number of times for animals but have been considered as comparatively rare phenomena. A comparison of the chromosomes of the rat and mouse, however, seems to indicate that these processes may have played a far more extensive role in vertebrate chromasome organization than has been heretofore thought. Such a shifting of material would not alter the genetic constitution of the animal, and hence would play no direct part in species formation, but it would throw added light upon the problem of the sterility which usually follows species crosses. That this sterility is based upon the failure of the chromosomes to mate up properly in maturation is the generally accepted and doubtless the correct explanation. With the facts just presented concerning the rat and mouse, we can understand better, perhaps, why this is the case. Just because two species look alike and are placed by systematists in the same genus, it does not follow that their chromosomes will be alike. On the contrary they may be very different as in the case immediately before us. This suggests that as a preliminary to species crosses a careful study should be made of the chromosome number and morphology in order to insure as far as this is possible that these are alike before crosses are attempted.

From the cytological standpoint the interpretation which has been given for the differences between the chromosomes of *Mus norvegicus* and *Mus musculus* lends added interest to an intensive study of the chromosomes of nearly related mammalian species in order to determine how frequent and extensive this shifting of material may have been, and how it is accomplished. Both phases are being investigated at this laboratory. At the present time it appears as possible to the writer that this change is accomplished, first, by a breaking up of the chromosomes into a larger number of smaller elements, and subsequently a fusion (without regard perhaps to previous association) of these smaller elements reducing the number again. This possibility is suggested by two sets of observations. In the first place in the wood rat common in the Austin region the chromosome number is high (about 54) and consists of relatively short chromosomes. There are no elements which correspond morphologically to the two largest pairs of chromosomes in *Mus norvegicus*. In the second place it has been observed that in the guinea pig the chromosome number is lower in prophase than in equatorial plate stages which can be interpreted as meaning either that we are witnessing in this form a breaking up of the chromosomes or a fusion of previously fragmented elements. Earlier, I was inclined to the former point of view, but the latter alternative is just as logical and seems now the more probable explanation in view of all the facts.

Since the chromatin furnishes directly or indirectly the physical substratum of the genes we should expect that the extensive redistribution of material which has taken place since the rat and mouse took their origin has entailed a rearrangement of the genes. If these two species carry approximately the same number of genes their distribution must be very different.

From a broader viewpoint the evidence which has been presented throws very grave doubt upon the idea that there is necessarily any extensive homology between the chromosomes of eutherian species in general or or even of closely related species. An extensive shifting of material between the chromsomes (such as has taken place in two closely related rodents) taken together with an inversion of a chromosome segment such as STURTEVANT and PLUNKETT have described would very quickly alter the genetic composition of any chromosome. The fact that the eutherian mammals so far studied all show a high number and a general similarity of seriation (in size) does not necessarily mean that there is any great similarity in the genetic make-up of, say, the largest chromosome. The

high number and perhaps the tendency towards a certain type of chromatin distribution may be dependent upon structural peculiarities of the eutherian cells and without any phylogenetic significance as far as the make up of the individual chromosomes is concerned.

SUMMARY

1. From considerations presented and discussed above we should expect the chromosomes of *Mus norvegicus* and *Mus musculus* to be similar. On the contrary they are very different.

2. In *Mus norvegicus* the diploid number is 42 and there are two pairs of large chromosomes nearly double the size of the next largest pair. In *Mus musculus* the diploid number is 40 and these form a closely graded series when placed in serial alinement.

3. The differences noted for the two species are too great to be accounted for on the basis of a breaking in two of a chromosome or the fusion of two pairs. It is concluded, therefore, that since the rat and mouse took their origin from a common stem there has been an extensive shifting of the chromatic material between non-homologous chromosomes, independently, in both species.

4. If the above conclusion is correct, added light is thrown on the problem of the sterility which results from species crosses and, furthermore, grave doubt must be entertained concerning the idea that there is necessarily any extensive homology between the chromosomes of different eutherian species.

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