goes a profound change in electronic structure on attachment to hemoglobin. The magnetic susceptibility of hemoglobin itself (ferrohemoglobin) corresponds to an effective magnetic moment of 5.46 Bohr magnetons per heme, calculated for independent hemes. This shows the presence of four unpaired electrons per heme, and indicates that the heme-heme interaction tends to stabilize to some extent the parallel configuration of the moments of the four hemes in the molecule. The bonds from iron to surrounding atoms are ionic in hemoglobin, and covalent in oxyhemoglobin and carbonmonoxyhemoglobin.

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[1] A. Gamgee, *Proc. Roy. Soc. London*, **68**, 503–512 (1901), and one or two more recent investigators have reported blood to be about as diamagnetic as water, without discovering the difference between arterial and venous blood.

[2] L. Pauling and C. D. Coryell, These PROCEEDINGS, **22**, 159 (1936).

[3] We shall discuss the structure of ferrihemoglobin and the ferrihemochromogens in a later paper.

[4] L. O. Brockway and P. C. Cross, *Jour. Chem. Phys.*, **3**, 828 (1935).

[5] L. Pauling, These PROCEEDINGS, **21**, 186 (1935).

[6] L. Pauling, *Jour. Am. Chem. Soc.*, **53**, 1367 (1931).

STRUCTURE AND ARRANGEMENT OF SALIVARY GLAND CHROMOSOMES IN DROSOPHILA SPECIES

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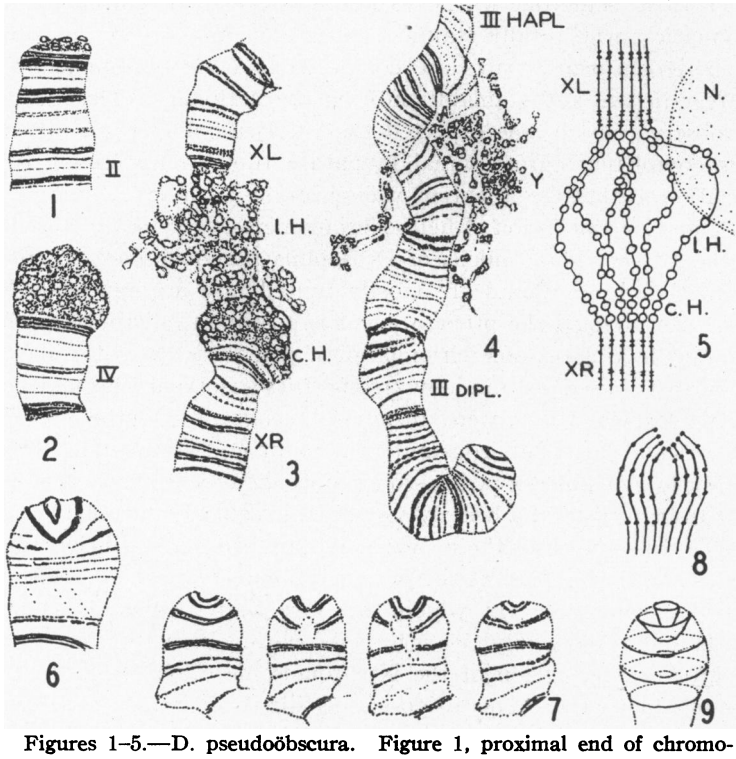
The salivary gland nuclei in Drosophila contain two different chromosome derivatives, the long chromosome strands which correspond to the euchromatic parts of prophase chromosomes, and the chromocenter which arises from the heterochromatic regions (Heitz[2]). While most investigators now agree that the euchromatic strands are composed of a number of closely united chromonemata, the homologous chromomeres of which form discs (aggregate chromomeres), there is no uniformity of opinion regarding the chromocenter. Painter[3] considered it an aggregate of accessory material into which each chromosome sends a thin achromatic strand. Muller and Prokofjeva[4] believe that these regions show the same

regular banding as the euchromatic parts. Koller[5] calls the chromocenter an "undifferentiated magma."

Observations in Chironomidae[6] led me to the conclusion that heterochromatic regions of salivary gland chromosomes are composed of the same number of chromonemata as the euchromatic strands, the difference between them being due to the structure of the single chromomeres. In euchromatic parts, totally staining dot-like euchromomeres are present, while heterochromatic parts are composed of heterochromomeres which are larger in size and stainable only on the periphery. On the basis of these observations it was expected that in Drosophila the chromocenter also is completely chromosomal in nature (not an apposed extraneous material), and that the apparent vacuolization is the expression of a close union and a partial fusion of heterochromomeres.

In the salivary gland nuclei of Drosophila pseudoöbscura the proximal parts of the chromosomes are mostly united into a compound chromocenter. By pressing the preparation it is possible to disrupt the chromocenter and to isolate some chromosomes. In these cases the parts of it are not distributed at random among these isolated chromosomes, as should be expected if it were an amorphous secretion product. Mostly each carries a part of it, constant in amount and structure. Two autosomes (III, IV) possess rather voluminous heterochromatic bases which appear to be formed of a heavily stainable, irregularly vacuolated material (Fig. 2). In some cases these "vacuoles" are arranged rather regularly in discs. That they represent the heterochromomeres, can be seen in more flattened chromosomes in which they are better isolated. The autosomes II and V show only one such heterochromomere discs each (Fig. 1). The chromocenter region of the X-chromosome differs from that of the autosomes in that it forms a looser, net-like body which often covers a part of the nucleolus surface. In contradistinction to the autosomes, the two limbs of the X, if separated by pressure, do not receive a constant amount of the chromocenter material. In most cases the longer limb has the larger part of it. In some nuclei the network forms a communication between both limbs (Fig. 3). The heterochromatic parts of the X consist of the same "vacuolized" granules, the heterochromomeres, as the bases of the autosomes; however, they are not apposed so closely, but form irregular, partly fused strings. Due to this, the heterochromomeres can be more easily recognized as single bodies. Judged from the different amounts of heterochromatin which each limb may receive, there is no predetermined breakage place in the X. Nevertheless, there is some evidence for determining the point of the spindle fibre attachment. The right limb possesses next to the euchromatin a more compact heterochromatic region which is equal in size and structure to the bases of chromosomes III and IV. According to the assumption that the V-shaped X-chromosome

is originally formed by the association of two rod-like chromosomes (the X and an autosome), the spindle fibre is probably located at the transition point between the loose and the compact heterochromatic regions. The only possible structural relation of the euchromatic and the heterochromatic parts is represented diagrammatically in figure 5.



Figures 1–5.—D. pseudoobscura. Figure 1, proximal end of chromosome II; figure 2, of chromosome IV. Figure 3, proximal part of *X*. Figure 4, III—*Y*-Translocation. Figure 5, diagram corresponding to figure 3; *XR, XL*, right and left limbs of *X*, *C.H.* and *L.H.*, compact and loose heterochromatin. The lines represent the chromonemata. The relation between the loose heterochromatin and the nucleolus (*N*) is indicated. Figures 6–9.—D. hydei. Figure 6, chromosome end with "ring"-bands. Figure 7, optical sections through the end of another chromosome with similar structure. Figure 8, diagram of the course of the chromonemata, corresponding to figure 7. Figure 9, same, showing the form of the terminal chromomere discs. Mag. 1–4: 1830×; 6, 7: 2430×.

The nature of the Y-chromosome in the salivary gland nuclei could be determined in translocations.[7] Figure 4 shows a case of a III-Y-translocation. The Y is completely of the same structure as the loose X-heterochromatin. It possesses no region which corresponds to the compact heterochromatin of *XR*.

In all cases where groups of two or three chromosomes remained together, the size of their common chromocenter was equal to the sum of the amounts present in the isolated chromosomes.

Observations on heterochromatic chromosome parts in mitosis of ganglion cells prove the correctness of the above interpretation. The Y is totally heterochromatic; the left limb of the X is heterochromatic in its proximal third, including the nucleolus-bearing secondary constriction. The right arm of the X and two of the autosomes (III, IV) show short heterochromatic regions, the heteropycnosis being not very conspicuous. The second and the fifth chromosomes seem to be completely euchromatic. (A single heterochromomere pro chromonema, therefore, cannot be detected in mitosis.)

The heterochromomeres as components of the heterochromatic parts of salivary gland chromosomes can be clearly seen in the X-chromosome of D. funebris and in the β-heterochromatin of D. virilis. In D. melanogaster, the maps of Bridges[8] show also that the heterochromatic regions (not designated as such) are formed by vesicular chromomeres. This is especially clear in the case of chromosome II, the continuity of which through the chromocenter could be proved. Here the heterochromomeres are quite regularly arranged in discs, while in the X and in III the omission of some letters indicates that the most proximal parts are more irregularly arranged, as in the species mentioned above. The description of Prokofjeva can be made to agree with that presented here on the assumption that she mistook the chromatic edges of the heterochromomere discs for the real aggregate chromomeres. The same more regular union of the heterochromomeres into discs is the case in the X-chromosome of D. hydei.

In D. melanogaster and D. pseudoöbscura the union of the chromosomes in a common chromocenter is, therefore, an expression of an attraction between the heterochromomeres. As I have pointed out,[6] the explanation of this attraction proposed by Prokofjeva:[9] homology between all heterochromomere regions, is not proved by any fact. The difference between the loose and the compact heterochromatin argues still more against this hypothesis. The union of the heterochromatic regions is better regarded as the expression of an attraction between non-homologous parts, characterized by a special kind of chromomeres.

D. funebris und D. hydei show a different behavior. In ganglion cells only the X- and Y-chromosomes are heterochromatic, the Y totally, the X of D. funebris in its proximal half, that of D. hydei in its shorter limb, and in a small area of the other limb adjacent to the spindle fibre constriction. The autosomes of funebris are completely euchromatic; in hydei one pair seems to possess a short heterochromatic (proximal?) end (Heitz[2]). Correspondingly, in the salivary gland nuclei only the X (and probably the Y which could not be identified with certainty) possesses

heterochromomeres, the autosomes appear to be completely euchromatic. In D. hydei the X shows the same behavior, but here also one long and probably the short chromosome contain a short heterochromomere region. Nevertheless, all chromosomes tend to be united by their proximal ends. This union, however, is not so intimate as in the species named above, and can be more easily destroyed by pressure.

The cause of this interchromosomal union of euchromatic parts can be shown by a study of the behavior of the distal free ends of the chromosomes. As I have described in Chironomus Thummi,[10] there exists a tendency of the free ends, to join with each other. In this species only union in pairs occurs in which all ends, except the left one of chromosome IV, take part at random. The same behavior can often be seen in all species of Drosophila. It depends, of course, on the degree of pressure during the preparation. In D. virilis, for instance, in 40 nuclei from a not very strongly pressed slide, 35% of the free ends of the 5 long chromosomes were united in groups of 2–4. Each of them takes part with the same frequency (12–15, $m = 13.6$), showing that the union is not a function of the heterochromomere-like terminal granules, present in the chromosomes I, III and V(=X) (Heitz[11]). The distal ends of the long chromosomes of D. funebris and D. hydei behave in the same manner. This terminal attraction must be effective also in their proximal free ends. The potentially equal attraction of proximal and distal ends to each other can be seen in cases in which chromosomes are only distally united. Chain formations ($Ip = Id - IId = IIp - IIIp = IIId$) occur rather frequently. Rare cases were found, in which the short chromosome was attached to the union point of several distal ends (possibly due to lagging during the last telophase). The difference in amount of proximal and distal union depends probably on the arrangement during the last telophase, in which the proximal ends of all chromosomes come close together, while the position of the distal ends, due to the different length of the chromosomes and the less exact orientation, is more variable.

The terminal attraction can explain two other peculiarities of the salivary chromosomes. The short chromosome in the species so far studied (comp. Bridges,[12] Tan[13]) is mostly curved, both ends being bent together or sticking to the chromocenter. It seems unnecessary to assume with Bridges the possible presence of a distal heterochromatic region in the fourth chromosome of D. melanogaster, as neither of the two criteria for such regions (heteropycnosis in prophases, heterochromomeres in salivary glands) agrees with this possibility. As all species show the same feature, it seems to be only a consequence of the small length of these chromosomes, not of their special structure. This would be expected, if the bending depends on the non-specific affinity between free ends.

The second fact, explainable by the terminal attraction, is the shape of

some chromosome ends. These often show ring-like terminal bands (Fig. 6). Heitz[11] took such figures as an argument for the peripheral arrangement of the chromonemata along an achromatic axis. Optical sections through these chromosome ends (Fig. 7), however, demonstrate that these "rings" can be followed through the interior of the chromosome. They form the edges of discs which are transformed from even plates into funnel-like structures. The course of the chromonemata must be as indicated in figure 8, the shape of the subterminal discs is represented diagrammatically in figure 9. The resulting intrachromosomal cleft can always be seen in such chromosome ends. Sometimes it opens out, especially in cases of excentric arrangement of the discs. These configurations can be understood on the assumption that the terminal chromomeres do not pair laterally, but tend to unite endwise. Simple mechanical conditions prevent the majority of the chromomeres from becoming arranged in this way. The result of this interaction is the special form of the terminal discs.[14] That only some chromosomes show such figures may be due to a competition between the lateral pairing attraction and the terminal affinity.

The special property of free ends is a fact. As an explanation for it, it may be assumed that the terminal chromomeres (or genes) are potentially bipolar but structurally connected only at one pole. The residual force of the free pole tends to become "saturated," which is possible only by union with another free pole (comp. Kossikov and Muller[15]).

Interchromosomal connections thus depend on two different forces: (1) the non-homologous attraction of heterochromomeres, (2) the non-specific affinity of terminal chromomeres. The first is effective in proximally heterochromatic V-shaped chromosomes of Drosophila (but not in the equally shaped chromosomes of Glyptotendipes[6]), the second in euchromatic ends. Both probably act together in the proximal union of partly heterochromatic rods. These forces tend to preserve the general telophase arrangement of the chromosomes.[16]

Painter[3] has raised the further question, whether there is a constant orientation of the chromosomes in regard to each other. He deduces this from the grouping in pairs of the chromosomes after breakage of the chromocenter. He finds that the fourth chromosome remains preferably together with an arm of the chromosome III. These observations, however, cannot decide the question, as the chromosome IV has its constant place in the center of the metaphase plate and consequently of the telophase group, preserved in the salivary gland nuclei. In D. pseudoobscura similar observations show no preferential grouping in pairs. Among 78 isolated pairs the 10 possible combinations of the 5 chromosomes occur with approximately equal frequencies (5–7–8–11; $m = 7.8$).

These results will be published in more detail elsewhere.

[1] Intern. Research Fellow of the Rockefeller Foundation.

[2] Heitz, E., *Zeits. Zellf. mikr. Anat.*, **19**, 720–742; **20**, 237–287 (1933).

[3] Painter, T. S., *Genetics*, **20**, 301–326 (1935).

[4] Muller, H. J., and Prokofjeva, A., *C. R. Acad. Sci. U. S. S. R.*, **1**, 658–660 (1935); Prokofjeva, A., *Ibid.*, **4** (1935).

[5] Koller, P. C., *Proc. Roy. Soc.*, **B118**, 371–397 (1935).

[6] Bauer, H., *Zool. Jahrb. Abt. allg. Zool.*, **56**, 239–276 (in press, 1936).

[7] Mr. C. C. Tan kindly supplied the stocks.

[8] Bridges, C. B., *Jour. Hered.*, **26**, 60–64 (1935).

[9] Prokofjeva, A., *C. R. Acad. Sci. U. S. S. R.*, **2**, 499 (1935).

[10] Bauer, H., *Zeits. Zellf, mikr. Anat.*, **23**, 280–313 (1935).

[11] Heitz, E., *Biol. Zentralbl.*, **54**, 588–609 (1934).

[12] Bridges, C. B., *Trans. Dynam. Development*, **10**, 463–473 (1935).

[13] Tan, C. C., *Proc. Nat. Acad. Sci.*, **21**, 200–202 (1935).

[14] These observations prove that in Drosophila as in Chironomus[10] the chromonemata fill the whole chromosome and are not only peripherally arranged. The second argument of Heitz[11] in favor of the latter view is based on so-called lateral attachments. Configurations like those described by Heitz are present also in D. funebris, D. hydei and D. pseudoöbscura. They represent short duplications (repeats). The rings figured by Heitz are only seldom to be seen and represent edges of the bent discs, as in the chromosome ends.

[15] Kossikov, K. V., and Muller, H. J., *Jour. Hered.*, **26**, 305–317 (1935).

[16] The presence of the terminal attraction accounts partly for the erroneous interpretation of a continuous spireme in early mitosis and meiosis. In the later stages of meiosis it manifests itself after the terminalization of the chiasmata in holding the dyads together.

FURTHER DATA ON LINKAGE IN RABBITS

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Castle and Nachtsheim[1] have shown that two genes (r_1 and r_2) for rex (short) coat in the rabbit are borne in a common chromosome, since they are linked with each other. An estimate was made by them of the crossover percentage based on breeding tests of 51 F_2 individuals derived from a repulsion cross, which indicated that the crossover percentage was about 10 or 12. Subsequently a race of homozygous double recessive individuals ($r_1r_1r_2r_2$) has been established by Castle. These produce only short-haired (rex) offspring when mated either with pure r_1 or with pure r_2 individuals. But if they are mated with F_1 individuals produced by a cross between pure r_1 and pure r_2 individuals, which F_1 animals themselves have a normal coat, a back-cross test for linkage is obtained which should give a more reliable estimate of the crossover percentage than an F_2 population of like size.