KUNGL. LANTBRUKSSTYRELSEN

Meddelanden från Statens undersöknings- och försöksanstalt för sötvattensfisket. Nr 23.
(Reports from the Swedish State Institute of Fresh-Water Fishery Research, Drottningholm.)

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Contents.

I. Introduction.

In the course of the last few decades immense advances have been made in cytological research, with the result that many problems of great importance have been solved or brought nearer their solution. As cytological research, for natural reasons, at first aimed mainly at a morphological analysis of different chromosome sets, many groups of organisms — both plants and animals — have been subjected to cytological examination. Nowadays, therefore, we know a good deal about the chromosome numbers and chromosome morphology in widely different groups of organisms.

Just as a system of classification preceded subsequent anatomical and physiological studies in botany and zoology, this broader chromosome research was soon followed by a minute study of the structure and chemistry of the chromosomes. Students of these branches of cytology naturally utilized the results of earlier research, in that, for example, they selected as their material chiefly organisms and chromosomes which, in view of their already known size or other characteristics, might be considered to be well adapted for study.

Certain groups of plants and animals, on the other hand, remained more or less unknown, because they had chromosomes which were too numerous or too small, or which were difficult to fix. To this very day there are certain groups which, to judge by a list of chromosome numbers, do not seem to invite deeper study. One of them is the lowest group of vertebrates — the fishes. Their position in the vertebrate series, however, seems to promise that interesting chromosome conditions will be found there. The genetic studies on aquarium fishes in fact indicate that these animals have a sex-chromosome mechanism deviating from the well-known *Drosophila* scheme.

OGUMA'S and MAKINO'S (1937) well-known list of chromosome numbers induced the author to devote special attention to this puzzling group of vertebrates. Especially the earlier studies on the salmons, referred to in this list, had made the author suspect that polyploidy had possibly played some part here during the phylogeny. Since MULLER'S investigations (1925), however, polyploidy has been considered to be practically ruled out as a principle for the evolution of bisexual animals. This question, therefore, seemed to be worth a deeper study.

Curiously enough, these investigations, which were commenced in 1939, yielded the result that all the earlier studies, broadly speaking, were in-

correct in regard to the chromosome numbers. But, though it was on those studies that the suspicion of polyploidy had been based, there is nevertheless reason to presume that polyploidy has actually played some part in the phylogeny of these fishes. In the study of this main question, also other cytological phenomena peculiar to these species were observed.

It is a pleasant duty to express here my sincere gratitude to Professor GERT BONNIER for all his helpfulness and the constructive criticism which he has offered to me in the course of this work.

I am also greatly indebted to Professor SVEN HÖRSTADIUS and Docent PER ERIC LINDAHL, Directors of the Department of Developmental Physiology and Heredity of the Wenner-Gren Institute.

I owe many thanks to my friend and colleague Dr. TORSTEN WICKBOM for numerous thorough-going discussions of current problems in this field ever since the commencement of the investigations, as well as to Miss KERSTIN BACHMAN and Miss MARIANNE TROILI for invaluable technical assistance.

Finally, Dr. GUNNAR ALM, Departmental Director at the Royal Agricultural Board has very kindly helped me to obtain material of various sorts. To me it has been particularly important to gain access to rare and useful specimens of sexually mature, several years old hybrid fishes.

My studies of the cytology of the Salmonoids began in 1939 at the Animal Breeding Institute, Wiad, Eldtomta and were in 1940 to 1943 transferred to the Wenner-Gren Institute in Stockholm. Both these institutions form part of the Stockholm University. The studies were concluded at the State Institute of Fresh-Water Fishery Research, Drottningholm.

The Faculty of Science of the Stockholm University and the Hierta-Retzius Foundation have contributed financially to the investigations. A grant from the British Council has facilitated the presentation of this work in English.

The chapters I—IV D have been translated by Mr GRENVILLE GROVE, the remaining parts by Mrs ULLA SCHÖTT.

Drottningholm, May 7th, 1945.

GUNNAR SVÄRDSON

II. Material and methods.

The principal material for this investigation consists of young embryos of all the Swedish fresh-water Salmonoids, namely *Salmo salar, trutta, alpinus* and *fontinalis, Coregonus lavaretus* and *albula, Thymallus thymallus* and *Osmerus eperlanus*. In addition, some hybrids were studied, namely *Salmo salar* × *Salmo trutta,* as well as that hybrid backcrossed to *Salmo trutta, Salmo trutta* × *S. alpinus* and *S. fontinalis* × *S. trutta.*

In view of the great technical difficulties previously encountered securing good chromosome pictures of these fishes, it seems desirable to describe the technical procedure in some detail.

Freshly fertilized roe was obtained from different fish-cultures. Such roe can quite well be transported by rail, provided that it is laid in a moist cloth bag in a wooden box containing moss. The rapidity of the embryonal development differs considerably in the different species, besides which it is dependent on the temperature. The roe was throughout allowed to develop in ordinary running tap-water, at a temperature of about 7—10 degrees centigrade. This temperature seems to be somewhat higher than that which is normal for the species (cf. OLOFSSON 1945): but a somewhat larger number of mitoses was thus obtained, which facilitated the studies.

For the study of the mitotic chromosomes, the author examined earlier embryonal stages than those dealt with by previous investigators on this subject. The most suitable stage for such study is passed through — at the said temperature — by salmon and other Salmo species approximately at the last stage of the third day of development: in the case of gwyniad the development is somewhat more rapid. As regards those species that spawn in the spring, *Thymallus* and *Osmerus,* the most suitable stage is during the second and first day, respectively.

Dissection of the embryonal disc proceeds in the following way. The egg is placed in an approximately 1 % NaCl solution, which prevents the yolk from coagulating. The egg is then securely held with a pair of pincers, and is punctured with a sharply ground scalpel, so that the yolk begins to flow out. The egg-shell is then cut out with sharp micro-scissors, so that the embryonal disc and the egg membrane are freed. The embryonal disc can then easily be detached from the egg membrane and the yolk; care, however, should be taken that no yolk goes along with it. The embryonal disc is then

transferred to a bath of NaCl solution, in order to wash away any yolk that may have adhered, which would impede the smearing. After some practice, this operation, from the placing of the living egg in the dissection bowl to the transfer of the embryonal disc to a fixation solution, will take only 20—30 seconds, thus permitting good fixation.

For fixation, the author used a 50 % solution of acetic acid, or aceto-carmine (BELLING 1926). If acetic acid is used for fixation, the embryonal disc must not lie in it longer than 10 minutes (as a maximum). In aceto-carmine, it should not lie longer than 15—20 minutes, after which the actual smearing should commence.

As a routine work, all the preparations were made permanent from the outset. The slide was coated with a thin film of albumin glycerine, hardened by being drawn for a few seconds through a burning flame. The cover-glass on its under-side was coated with a thin film from the secretion of the sebaceous glands.

The embryonal discs were laid on the slide and the prepared cover-glass was laid over it, whereupon the actual smearing was made with a small spatula. The degree of hardness of the smearing must be judged from case to case: the smaller the nuclei, the harder, of course, the pressure which they will stand. As a rule, however, the normal position of the spindle is disturbed and, if the spindle is to be studied, the pressure must be very weak. Unfortunately, the subsequent removal of the cover-glass will thereby be impeded.

After the removal of superfluous acetocarmine with filter-paper, the preparation is ready for preliminary examination in the microscope, when it can as a rule be determined (by shading) whether the suitable stage has been found, or whether the nuclei are too large or too small. Detailed chromosome studies, however, cannot be made on these preparations, as the colour is too faint.

If the preparation is found to be worth further study, it is laid in a 96 % solution of spirit, where it is left until the cover-glass loosens. Previous drying in air for half-an-hour or an hour will do no harm and will facilitate the removal of the cover-glass. This is in fact the most critical point in the procedure and, if the pressure has been too weak, or the albumin glycerine film too dry (stale), some part of the preparation may be carried away on the lifting of the cover-glass.

After the removal of the cover-glass, which as a rule has taken place on the day after the arrangement of the preparation, the definitive dyeing in acetocarmine is started at a high temperature. For the author's preparations, he chiefly used a thermostat at 48 degrees centigrade. After three to five

hours the preparation is completely dyed. It should be noted, however, that testicle material — which in other respects can be treated in exactly the same way —, requires considerably less time, as a rule at most an hour.

After dyeing, the preparation is transferred to spirit and passes through the ordinary series of increasingly higher grades of alcohol up to xylol, whereupon it is treated with Canada balsam. These preparations are very durable and after three to four years they have scarcely paled.

Comparative tests have shown that material treated according to one of the usual section methods regularly yields inferior pictures: this applies also to testicle material.

The optic system adopted was a Zeiss binocular microscope with a Leitz fluorite objective 112 ×, having an aperture of 1.32. Compensating eyepieces with a magnification of 15 × or 20 × were employed, besides a Leitz camera lucida.

III. Previous literature.

The salmons, as we know, have long been a favourite object of embryological study, presumably owing to the comparative ease with which freshly fertilized roe can be procured from fish-cultures. Many investigators who have studied the problems of fertilization and embryonal development have therefore been able incidentally to make observations also on the chromosomes of the salmons.

In the well-known lists of chromosome numbers (OGUMA and MAKINO, 1937, MC CLUNG, 1940), it is stated that BÖHM was first to mention chromosomes, when in 1891 he was studying the fertilization of *Salmo fario,* now called *Salmo trutta (fario).* It should be noted, however, that as far back as 1887 SCHWARZ had studied the embryonal cleavage of the same species and had found coloured »Schleifen», which he homologized with the chromosomes studied by FLEMMING and others. Repudiating the view of earlier authors that it is useless to try to count these chromosomes, he determined their number, when in »stellar form», at 48. He thought that it showed curious correspondences with the numbers 24 and 12 found by FLEMMING and others, especially in certain amphibia.

BÖHM (1891), studying the course of fertilization in *Salmo trutta,* observed, 20 minutes after the external fertilization, the first metaphase of the maturation divisions. Here he thought he could distinguish 12 rod-shaped elements. In the formation of the first polar body he still saw 12 stainable elements. The same was the case in the formation of the second polar body. Thus, according to BÖHM, the haploid chromosome number in *Salmo trutta* is 12, that is, merely half the figure found by SCHWARZ. SCHWARZ' work was unknown to BÖHM.

BLANC (1894) likewise studied the fertilization, but on »*Trutta lacustris*». This species too presumably corresponds to *Salmo trutta.* Although he considered himself unable to state the number of chromosomes, he shows two pictures (his Figs. 2 and 3) which in all probability represent the metaphase of the first division, viewed from the side and from above. Both these pictures are so indistinct that it is not possible to count the number of chromosomes, but the present writer estimates them roughly at about 40. Especially the picture of the metaphase viewed from the side gives the impression of bivalents: and though several of BLANC'S statements in other respects have

required revision, his view of the chromosome number seems to have been fairly correct.

BEHRENS (1898) studied the fertilization of *Salmo fario, i. e. trutta,* and *Salmo irideus,* the rainbow trout. This author, who in the main supported BÖHM against BLANC, seems to have been thus induced to agree with BÖHM also in regard to the number and form of the chromosomes. He shows several pictures of the two species, where 12 chromosomes bent at an angle and in anaphase halves are distinctly seen. He also claims to have several times been able to count them with considerable certainty. Thus, according to BEHRENS, the diploid number both for *Salmo trutta* and *Salmo irideus* is 24.

OPPERMAN (1913), studying how radium-irradiated spermatozoa affected the course of the fertilization in *Salmo trutta,* observed in the embryos also the mitoses and tried to count its chromosomes. These mitoses were examined on the radium-treated material, which, according to OPPERMAN, consisted of a haploid (maternal) embryo. He states (*op. cit.* p. 316): »Während in normalen Teilungsfiguren eine Bestimmung der Zahl der Chromosomen wohl unmöglich ist, konnte in einigen Fällen bei den Versuchsobjekten die Zahl mit einem hohen Grade von Wahrscheinlichkeit auf 12 festgestellt werden». As OPPERMAN'S number, of course, applies to the haploid set, his figures are in complete correspondence with those of BÖHM and BEHRENS.

When MRSIC (1923) made his well-known study of the sex ratio and sex differentiation in *Salmo irideus,* he also touched on the question of the chromosome number (p. 168). Referring to the recorded number 12 for *Salmo trutta,* he states that it should be applicable also to *Salmo irideus.* As he was studying somatic mitoses, he ought to have obtained the number 24 (BÖHM and BEHRENS), but evidently he had not made a thorough study of those investigations.

Summing up, the following numbers have thus been recorded in the literature as the diploid number of chromosomes in *Salmo trutta:* 12 (MRSIC), 24 (BÖHM and BEHRENS, OPPERMAN), 48 (SCHWARZ) and — not determined, but roughly estimated —, 80 (BLANC). It should be evident from this survey that these figures are nowadays merely of historical interest. They have been summarized and examined in order to give support to the view that they need no longer occupy a place and claim attention on lists of chromosome numbers.

The first modern study on the chromosomes of the salmons was that published by the Russian cytologist, PROKOFIEVA, in 1934. She had studied *Salmo salar, Salmo fario (i. e. trutta), Coregonus lavaretus baeri* as well as the hybrids *Salmo salar* × *Salvelinus fontinalis* and *Coregonus lavaretus baeri* × *Salvelinus fontinalis.* The diploid number of the chromosomes was

determined at 60 for *Salmo salar,* 80 for *Salvelinus fontinalis,* 84 for *Salmo trutta* and 80 for *Coregonus.* Her material consisted of embryos 5—10 days old after fertilization. PROKOFIEVA'S chromosome morphology will be discussed in connection with my own results.

MAKINO (1937) studied *Oncorrhynchus keta Walb,* a salmon closely related to *Salmo salar.* His material consisted of newly hatched embryos, and his chromosome studies were made on mitoses of the primordial germ cells. The diploid number was determined by him at 74.

The latest study — so far as I am aware —, on the Salmonoid chromosomes was made by POMINI (1939). He studied four *Salmo* species, namely *fario, carpio, lacustris* and *marmoratus.* The systematic position of these forms had long been very obscure and POMINI had been convinced by his morphological studies that they must be regarded as different species. He desired, however, to confirm this view by a cytological investigation. His material consisted of embryos 3—5 days old. With the sectioning technique adopted, however, his pictures were so bad that he was unable to determine the chromosome number with certainty in any single case. *Salmo marmoratus* and *fario* corresponded with one another as well as with PROKOFIEVA'S number: 84. He found, however, that *Salmo lacustris* deviated from this number, having about 70 chromosomes, as also *S. carpio,* the diploid number of which he considered to be about 96. Being unable to make any independent morphological studies, he could merely confirm PROKOFIEVA'S statements regarding the existance of V-shaped and rod-shaped chromosomes.

The above review indicates that even modern studies on the chromosomes of the salmons have presented great difficulties. In view of the unusually large number of chromosomes and their tendency in the metaphase plate to arrange themselves in groups or with their ends towards one another (compare the following pictures of *Salmo alpinus,* in which species this phenomenon is particularly marked), it is difficult even to determine the chromosome numbers. This is complicated by the fact that the chromosome number sometimes varies owing to non-disjunction.

The difficulties in studying the meiosis are still greater and no previous investigations whatever on this subject seem to be available.

IV. Results.

A. Some general cytological features of Salmonoid chromosomes.

One of the most important discoveries in modern cytology is that the chromosome mechanism appears to be similar in widely different groups of plants and animals. Thus, by studying a group of plants, we can draw conclusions regarding hitherto puzzling phenomena in animals, and *vice versa*.

Nevertheless, certain organisms show peculiar features which we do not find in other groups. The salivary gland chromosomes of *Diptera* are perhaps the best example. As was the case with these chromosomes, the future will probably show that cytological phenomena which were at first considered to be specific of a certain group can — in a modified form — be found, and explained, in other groups of organisms.

A cytological study of the Salmonoids must be confined mainly to the mitosis, in view of the large number of chromosomes and the minute size of the nuclei at meiosis. At mitosis, on the other hand, — at any rate during a limited stage —, the nuclei are large and relatively easy to fix. The difficulties previously encountered even in simple determinations of chromosome numbers have, thanks to modern smear technique, now been very considerably reduced. By way of introduction, certain cytological features in these fishes will be dealt with. Whether these characters are confined to the Salmonoids, the future will show. They are presumably distinctive of the mitosis in many forms, though the mitosis, to which comparatively little interest has been devoted, is relatively little known.

1. Chromosome volume changes.

Introductory. The chromosome volume is not the same in every cell of an organism, nor always the same in two individuals of the same species, nor indeed within a certain cell nucleus. We already know various examples of variations in size, as regards the length and breadth of the chromosomes, viewed in the microscope. It may be of some value to distinguish between the *intra-nuclear* and the *inter-nuclear* variation in size.

The intra-nuclear variation in size is the best known. At every mitosis and meiosis the chromosomes passes through a series of external changes in form, which are now generally viewed as a spiralization cycle. Since 1880, when BARANETSKY discovered the spiral structure of the chromosomes at the meiotic metaphase, these structures have been busily studied, and our knowledge of the normal spiralization cycle has been eked out especially by Japanese investigators as well as by C. D. DARLINGTON and his school. Thus, from prophase to metaphase, the chromosome passes through a spiralization, which renders it shorter and broader. This is now a well-known rule, though there are isolated exceptions where modifications occur (DARLINGTON 1937, GEITLER 1938 a).

The inter-nuclear variation in size, on the other hand, extends to all the differences between different nuclei. In many cases, as DARLINGTON and UPCOTT (1939) have tried to show, this variation may likewise be attributed to spiralization of different degree. In other cases, on the other hand, other factors may come into play. HANCE (1926) found that the chromosomes in tissue cultures of chick embryos were larger than *in situ*, which GEITLER (1938 a) ascribes to better nutritive conditions. There are also genetic differences, as, for example, when the chromosomes in the one sex are larger than in the other, when the chromosome size changes on hybridization, and so on. Many investigators have incidentally observed the variation in chromosome size, but have rarely tried to explain it or even to distinguish between changes in length and variations in breadth. The earlier literature on the subject has been summarized by DARLINGTON (1937), GEITLER (1938 a, 1940 a, 1940 b, 1941) and TISCHLER (1942), to whom the reader is referred. Recent data regarding variations in chromosome size, but without an attempt at explanations, will be found also in PARTHASARATHY (1939), SRINATH (1939), GRAFL (1940), DANGEARD (1941), STEIN (1942) etc. In recent times also genetic variations in size have been shown by KLINGSTEDT (1939), FEDERLEY (1939), DARLINGTON (1941 b) and TOGBY (1943). Some earlier statements to the effect that differences in chromosome size in two closely associated species may be retained in the hybrid between them — which statements according to DARLINGTON (1937) could not be verified by subsequent experiments —, have, however, again been confirmed by HÅKANSSON (1943) and LEVAN (1944 b). The phenomenon is still unexplained.

In haploid nuclei the chromosomes are often larger than in diploid (FANKHAUSER 1934). This phenomenon has been studied also by PÄTAU (1936) and COOPER (1939), who found that it was due to the cross-sectional area of the spindle. When the metaphase area of the spindle in successive divisions is reduced, the chromosomes in diploid nuclei become more crowded than in

haploid nuclei, thus diminishing both in length and breadth. COOPER considers therefore that the spindle area »controls» chromosome size, but the mechanism is very obscure.

The problem of the reasons for the internuclear variations in chromosome size has not been discussed with such interest as might have been expected. DARLINGTON (1937, p. 56), as regards the changes where spiralization seems to be ruled out as an explanation, has confined himself to referring to hydrogen-ion differences and attributes the variations in size »to differences of dispersion of the permanent material (genes) in the resting nucleus and in the chromosomes rather than to a different degree of proliferation of these materials». GEITLER, on the other hand, has devoted more attention to this question and, after reporting some examples of how the chromosomes slowly diminish in size in successive divisions, states (GEITLER 1940 b p. 246): »Im ganzen ergibt sich, dass das Problem der Chromosomengrösse noch ungelöst ist . . . Es scheint daher vorläufig ohne die Annahmne nicht auszukommen zu sein, dass das Chromonema selbst wachstumsfähig ist und sich mit mehr oder weniger Substanz beladen kann; allerdings bereitet diese Annahme in Hinblick auf die derzeitigen theoretischen Vorstellungen vom miszellaren Feinbau der Chromonemata Schwierigkeiten . . .»

Own results. The very first stages of cleavage in the Salmonoid embryos have not been studied. As previously mentioned, it is only during a brief stage of the embryonal cleavage that good chromosome pictures can be obtained — merely for about 12 hours. Before that time the nuclei are too large for smearing and the embryos contain merely relatively few mitoses. After this stage, on the other hand, the nuclei are too small, so that chromosomes are liable to clump and give bad pictures. From the first division of the fertilized egg up to the meiosis of the mature individual, the nuclei become increasingly smaller and the chromosomes with them. G. HERTWIG (1939), in studies of the divisions of the fertilized egg of rats, has found that the first nuclei at each division are reduced to half. From this he concluded that the chromosomes have a »metameri» *i. e.* are built up of many chromonemata. GEITLER (1940—41) on several occasions has critized HERTWIG'S view; nor do the embryonal cleavages studied by me give any support to it.

In the salmon the very largest chromosomes during the third day of development in the prometaphase are about 15 microns long and about 1.5 microns broad (in acetocarmine-swelled material). With the same fixation the largest chromosomes in the metaphases of the spermatogonia are about 3 microns in length and narrower than 1 micron. Such variations in size in the ontogeny are certainly common in animals, and induced the earlier investigators

(Mc Clung 1905, Meek 1913) to stress the value of relative measurements of size between the chromosomes. Darlington's (1937 p. 28) statement that the chromosomes in the mitotic metaphase »are as a rule invariable at this stage throughout the organisms» thus does not apply to animals and presumably not to plants either (cf. Geitler 1941 and Tischler 1942).

That the chromosomes in haploid nuclei are often larger than in diploid (see above) I have been able to control in the Salmonoids. I have, however, been unable to make any measurements of the area of the spindle, as the dimensions of the spindle are disturbed in the smearing, which was often rather rough. That the chromosomes of the brown trout *(Salmo trutta)* are enlarged in the F1-hybrid with salmon *(Salmo salar)* which has larger chromosomes, has likewise been ascertained (*vide infra* p. 56).

Even in studying the chromosome morphology of the different species, the observation could be made that the changes in size did not seem to be quite proportional, whence a closer study of this question became desirable.

Thanks to some good metaphase plates of *Salmo (Salvelinus) alpinus,* where the smearing was so intense that the chromosomes lay almost flat, measurements of the chromosomes could be carried out. With Leitz' camera lucida each individual chromosome was drawn in the centre of the field of vision of the microscope at a working magnification of 3 360 ×. In this way perspective displacement with attendant errors in measurement could be reduced. On the drawings thus made, where the chromosomes, thanks to the magnification with the camera lucida, were reproduced at a magnification of 5 100 ×, the length and breadth were reckoned. The length was computed according to the longitudinal axis of the chromosome and the breadth was measured at three different places, whereupon an average of these measurements was adopted. The sources of error in the measurement of the breadth are naturally rather considerable. By way of experiment, also the slight difference between the highest and lowest point of each chromosome was measured with the aid of a graded micrometer, whereupon the correction was computed for the real length. It was found, however, that the differences were so slight that this laborious procedure could be abandoned. My arrangements may seem to be simple, as compared with the apparatus used by Powers (1942) for exact measurements, but the more favourable nature of my material, as the chromosomes were lying flat, should be taken into account. His object — the observation of possible differences in structure between different species and subspecies, in fact required extremely precise measurements.

All the measurements obtained have been summarized in Table 1. The plates selected for closer study of the variation in size consist of one pro-

Table 1. Length and breadth in microns of all 80 chromosomes in four different metaphase plates of Salmo alpinus. Shortening percentages. See text.

No.	A		B		C		D		A–C	A–D	B–C	B–D
	length	breadth	length	breadth	length	breadth	length	breadth	%	%	%	%
1	13.52	0.43	13.92	0.76	11.47	0.67	11.27	0.43	15.2	16.6	17.6	19.0
2	12.15	0.39	13.92	0.75	11.18	0.67	10.98	0.45	8.0	9.6	19.7	21.1
3	11.96	0.55	13.14	0.74	10.39	0.69	10.29	0.53	13.1	14.0	20.9	21.7
4	11.96	0.49	12.94	0.73	9.61	0.65	10.00	0.43	19.6	16.4	25.7	22.7
5	10.78	0.45	12.55	0.73	9.51	0.59	9.61	0.43	11.8	10.9	24.2	23.4
6	10.39	0.49	11.96	0.78	9.02	0.69	9.41	0.49	13.2	9.4	24.6	21.3
7	10.29	0.51	11.57	0.69	8.63	0.75	9.41	0.47	16.1	8.6	25.4	18.7
8	10.20	0.41	11.57	0.75	8.53	0.63	9.41	0.39	16.4	7.7	26.3	18.7
9	10.20	0.55	11.18	0.75	8.43	0.67	9.31	0.49	17.4	8.7	24.6	16.7
10	10.00	0.45	10.98	0.73	8.24	0.63	8.73	0.53	17.6	12.7	25.0	20.5
11	9.22	0.41	10.00	0.76	8.24	0.67	8.53	0.41	10.6	7.5	17.6	14.7
12	9.12	0.39	9.61	0.71	7.84	0.75	7.84	0.45	14.0	14.0	18.4	18.4
13	8 82	0.47	9.51	0.75	7.35	0.71	7.25	0.43	16.7	17.8	22.7	23.8
14	8.04	0.47	9.12	0.75	7.06	0.73	7.25	0.43	12.2	9.8	22.6	20.5
15	8.04	0.53	8.53	0.75	7.06	0.69	7.16	0.39	12.2	10.9	17.2	16.1
16	7.94	0.45	8.24	0.76	6.47	0.71	6.86	0.47	18.5	13.6	21.5	16.7
17	12.75	0.39	11.18	0.67	8.14	0.63	7.65	0.39	36.2	40.0	27.2	31.6
18	12.55	0.47	10.49	0.69	7.94	0.63	7.16	0.41	36.7	42.9	24.3	31.7
19	9.02	0.59	9.22	0.63	6.96	0.65	7.06	0.39	22.8	21.7	24.5	23.4
20	8.73	0.41	8.63	0.67	6.67	0.73	6.76	0.39	23.4	22.6	22.7	21.7
21	7.84	0.43	8.33	0.65	5.88	0.73	6.76	0.39	25.0	13.8	29.4	18.8
22	7.45	0.41	8.24	0.75	5.78	0.71	6.57	0.43	22.4	11 8	29.9	20.3
23	7.06	0.43	7.65	0.76	5.59	0.59	6.37	0.45	20.8	9.8	26.9	16.7
24	6.86	0.43	7.55	0.67	5.49	0.65	6.18	0.39	20.0	9.9	27.3	18.1
25	6.67	0.37	7.45	0.75	5.49	0.59	5.98	0.41	17.7	10.3	26.3	19.7
26	6.47	0.39	7.45	0.67	5.39	0.65	5.78	0.39	16.7	10.7	27.7	22.4
27	6.47	0.41	7.25	0.78	5.29	0.65	5.59	0.39	18.2	13.6	27.0	22.9
28	6.47	0.41	7.25	0.75	5.29	0.71	5.49	0.39	18.2	15.1	27.0	24.3
29	6.37	0.41	7.06	0.78	5.29	0.65	5.49	0.45	17.0	13.8	25.1	22.2
30	6.37	0.41	7.06	0.82	5.10	0.69	5.49	0.45	20.0	13.8	27.8	22.2
31	6.37	0.47	6.96	0.78	5.00	0.63	5.49	0.37	21.5	13.8	28.2	21.1
32	6.27	0.43	6.76	0.78	4.90	0.63	5.49	0.35	21.9	12.4	27.5	18.8
33	6.27	0.45	6.76	0.67	4.90	0.71	5.29	0.39	21.9	15.6	27.5	21.7
34	6.27	0.43	6.67	0.61	4.90	0.69	5.29	0.37	21.9	15.6	26.5	20.6
35	6.27	0.49	6.67	0.69	4.90	0.69	5.20	0.37	21.9	17.1	26.5	22.0
36	6.18	0.43	6.67	0.63	4.90	0.61	5.10	0.39	20.7	17.5	26.5	23.5
37	6.18	0.43	6.59	0.65	4.90	0.65	5.10	0.39	20.7	17.5	25.6	22.6
38	6.08	0.45	6.57	0.67	4.71	0.63	5.00	0.41	22.5	17.8	28.3	23.9

No.	A		B		C		D		A–C	A–D	B–C	B–D
	length	breadth	length	breadth	length	breadth	length	breadth	%	%	%	%
39	5.98	0.47	6.47	0.65	4.71	0.63	5.00	0.43	21.2	16.4	27.2	22.7
40	5.88	0.47	6.47	0.67	4.71	0.69	5.00	0.39	19.9	15.0	27.2	22.7
41	5.88	0.51	6.47	0.76	4.71	0.61	4.90	0.39	19.9	16.7	27.2	24.3
42	5.88	0.47	6.47	0.67	4.71	0.67	4.90	0.39	19.9	16.7	27.2	24.3
43	5.78	0.43	6.18	0.69	4.71	0.78	4.90	0.39	18.5	15.2	23.8	20.7
44	5.78	0.39	6.08	0.75	4.71	0.69	4.90	0.41	18.5	15.2	22.5	19.4
45	5.78	0.53	6.08	0.63	4.71	0.67	4.80	0.43	18.5	17.0	22.5	21.1
46	5.69	0.59	6.08	0.69	4.61	0.57	4.80	0.41	19.0	15.6	24.2	21.1
47	5.69	0.55	5.98	0.78	4.61	0.59	4.80	0.39	19.0	15.6	22.9	19.7
48	5.59	0.39	5.98	0.80	4.61	0.57	4.71	0.37	17.5	15.7	22 9	21.2
49	5.59	0.43	5.88	0.73	4.61	0.71	4.71	0.35	17.5	15.7	21.6	19.9
50	5.59	0.41	5.78	0.69	4.51	0.61	4.71	0.41	19.3	15.7	22.0	18.5
51	5.59	0.43	5.69	0.67	4.51	0.69	4.71	0.43	19.3	15.7	20.7	17.2
52	5.49	0.55	5.69	0.75	4.51	0.67	4.51	0.39	17.9	17.9	20.7	20.7
53	5.49	0.51	5.69	0.67	4.51	0.65	4.51	0.39	17.9	17.9	20.7	20.7
54	5.49	0.49	5.69	0.73	4.51	0.57	4.51	0.39	17.9	17.9	20.7	20.7
55	5.49	0.43	5.59	0.65	4.51	0.59	4.51	0.35	17.9	17.9	19.3	19.3
56	5.49	0.45	5.49	0.65	4.51	0.73	4.51	0.43	17.9	17.9	17.9	17.9
57	5.49	0.47	5.49	0.75	4.51	0.65	4.51	0.43	17.9	17.9	17.9	17.9
58	5.49	0.43	5.49	0.73	4.41	0.59	4.41	0.45	19.7	19.7	19.7	19.7
59	5.39	0.41	5.49	0.61	4 41	0.59	4.41	0.41	18.2	18.2	19.7	19.7
60	5.39	0.53	5.49	0.76	4.41	0.71	4.41	0.39	18.2	18.2	19.7	19.7
61	5.39	0.47	5.39	0.75	4.41	0.67	4.31	0.47	18.2	20.0	18.2	20.0
62	5.29	0.51	5.29	0.69	4.31	0.65	4.31	0.39	18.5	18.5	18.5	18.5
63	5.20	0.45	5.29	0.65	4.31	0.69	4.31	0.41	17.1	17.1	18.5	18.5
64	5.20	0 43	5 20	0.65	4.31	0.69	4 22	0.33	17.1	18.8	17.1	18.8
65	5.10	0.39	5.10	0.82	4.31	0.75	4.22	0.43	15.5	17.3	15.5	17.3
66	5.10	0 37	5.10	0.76	4.12	0.65	4.22	0.47	19.2	17.3	19.2	17.3
67	5.10	0.39	5.10	0.73	4.12	0.73	4.12	0.41	19.2	19.2	19.2	19.2
68	4.90	0.49	5.10	0.69	4.12	0.59	4.12	0.39	15.9	15.9	19.2	19.2
69	4.90	0.41	5.10	0.63	4.12	0.65	4.12	0.39	15.9	15.9	19.2	19.2
70	4.71	0.37	5.10	0.75	4.12	0.69	4.12	0.39	12.5	12.5	19.2	19.2
71	4.71	0.51	5.00	0.75	4.02	0.73	4.02	0.39	14.6	14.6	19.6	19.6
72	4.61	0.41	4.90	0.67	4.02	0.71	4.02	0.37	12.8	12.8	18.0	18.0
73	4.61	0.41	4.90	0.76	4.02	0.61	4.02	0.45	12.8	12.8	18.0	18.0
74	4.51	0.39	4.90	0.69	3.92	0.65	4.02	0.37	13.1	10.9	20.0	18.0
75	4.51	0.41	4.80	0.73	3.92	0.65	3.92	0.43	13.1	13.1	18.3	18.3
76	4.51	0.55	4.71	0.76	3.82	0.73	3.92	0.39	15 3	13.1	18.9	16.8
77	4.41	0.43	4.61	0.78	3.73	0.69	3.92	0.41	15.4	11.1	19.1	15.0
78	4.31	0.49	4.61	0.67	3.73	0.59	3.92	0.39	13.5	9.0	20.4	15.0
79	4.31	0.41	4.31	0.78	3.63	0.71	3.73	0.43	15.8	13.5	15.8	13.5
80	4.12	0.45	3.63	0.67	3.53	0.67	3.43	0.41	14.3	16.7	2.8	5.5

metaphase (A), two mid-metaphases (B and C) and one metaphase where the anaphase separation had already begun (D). The intranuclear variation would thus be illustrated by the comparison A—C—D, and the internuclear by the comparison B—C. The metaphases A and B are derived from the same embryo; B and C, on the other hand, from two others. All of them, however, were simultaneously fixed and in every respect identically treated.

The intranuclear change may be said to be a shortening and thickening. On the other hand, the internuclear change (the term is, of course, used in its broadest sense, thus involving also successive generations of the same mother-nucleus) implies that the chromosomes are shortened and become narrower. The same nucleus, however, could not, of course, be fixed more than once and a study of the intranuclear variation must thus be based on a comparison between different nuclei of as similar age as possible. In this there is, of course, a source of error, as the cell divisions of the Salmonoids are not synchronous and the nuclei may thus have different numbers of generations behind them. The intra- and internuclear variation will therefore partly coincide, but thanks to the essentially different change in the breadth of the chromosome, which increases in one case, whilst it diminishes in the other, the two types of variation can rather easily be kept distinct. Several years' training also makes it rather easy to judge the mitosis stage (pro-, mid- or late metaphase) of a nucleus, the constrictions *(vide infra)* being also of good assistance.

»In most organisms contraction of the chromosome reaches its maximum at the latest stages of prophase» says DARLINGTON (1937 p. 25) regarding the mitosis. This is not the case with the salmons. Here, on the contrary, the chromosomes enter a metaphase and orient themselves in the spindle into a typical plate whilst the chromosomes are still far from maximal contraction, which is not attained until before the beginning of the anaphase. Thus, while they lie in the metaphase plate they undergo highly remarkable changes in size. It is only these changes in size *within the metaphase* that have been studied.

The metaphase plates A and C probably belong to the same nuclear generation, whereas B and D have one or possibly two generations less behind them. The breadth of the »chromosome» in D comprises merely the breadth of the chromatid and thus corresponds merely to half of the others. The diploid 80 chromosomes in *Salmo alpinus* have been divided (in Table 1) into groups of 16. The first group comprises 16 V-shaped chromosomes, whereas all the others are rod-shaped, but these too have been divided into groups of 16 in order to facilitate a statistical examination of the results obtained. The chromosomes have been arranged in numerical order from

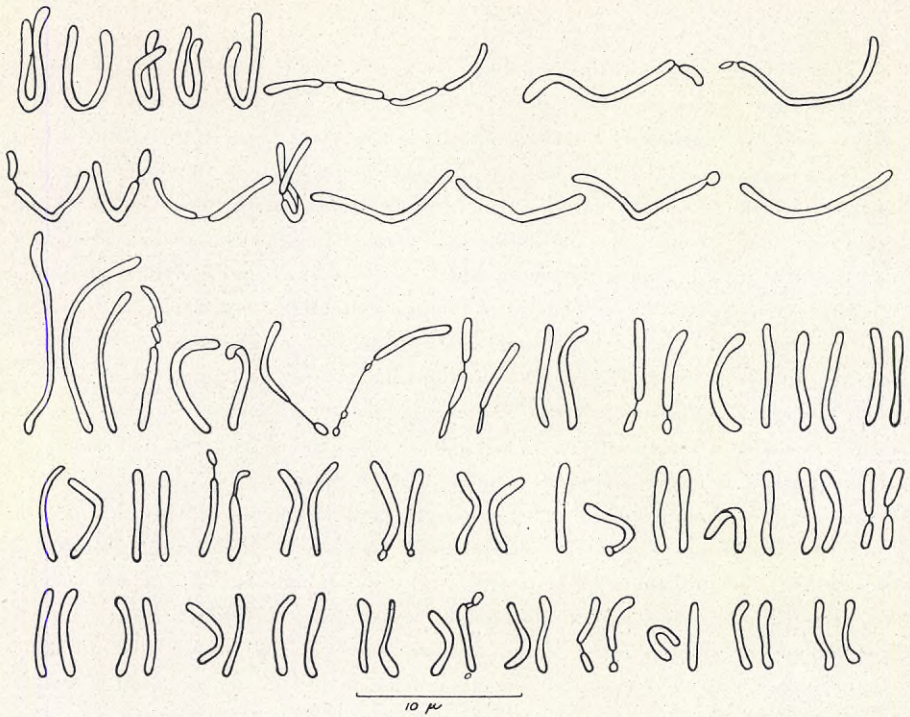


Fig. 1. Salmo alpinus. Mitotic chromosomes. Prometaphase (A).

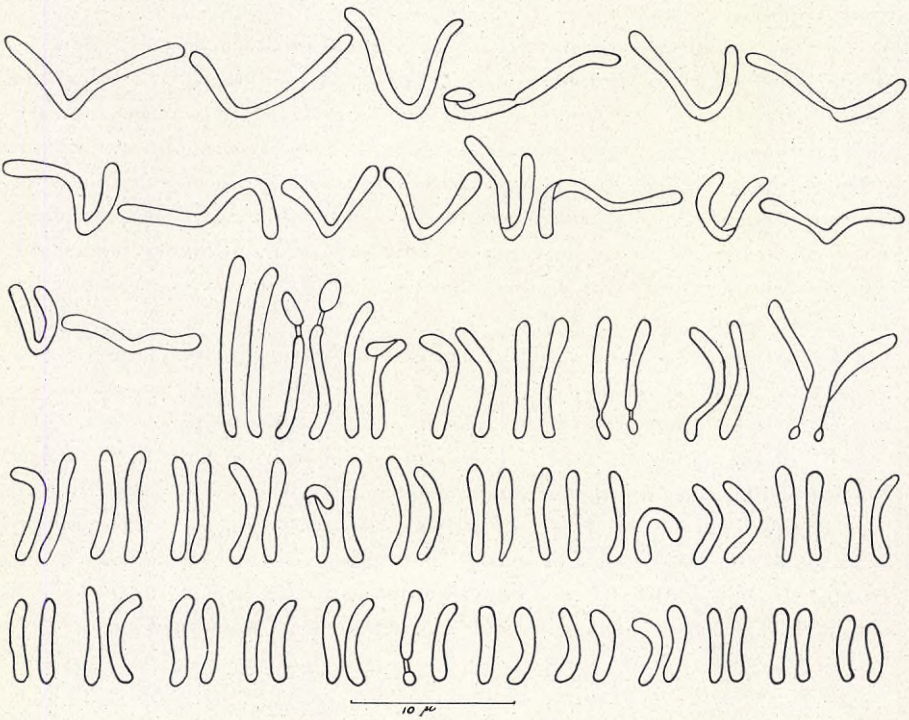


Fig. 2. Salmo alpinus. Mitotic chromosomes. Mid-metaphase (B).

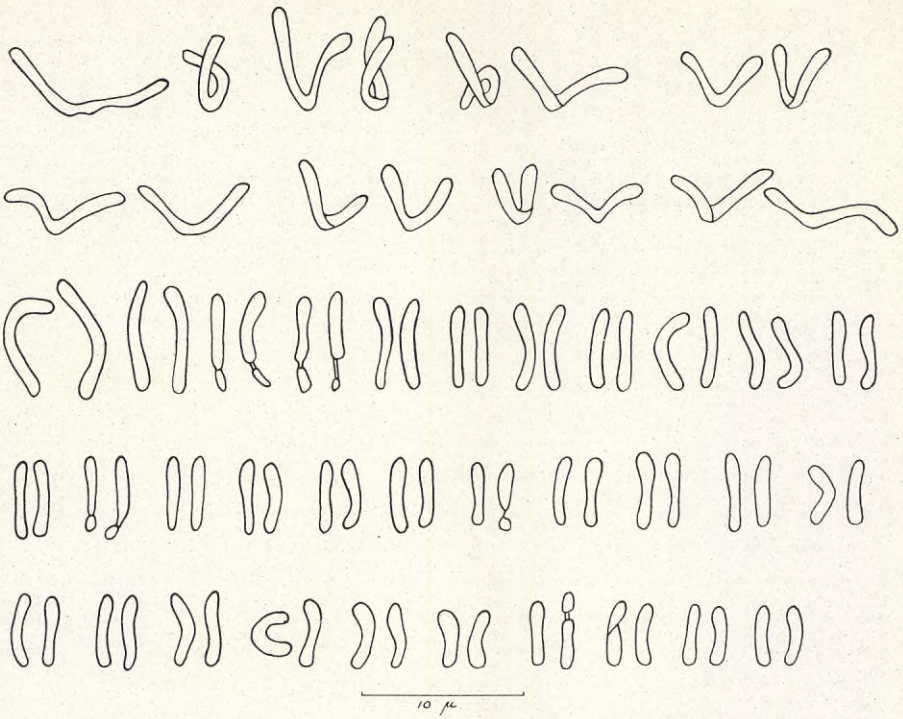


Fig. 3. Salmo alpinus. Mitotic chromosomes. Mid-metaphase (C).

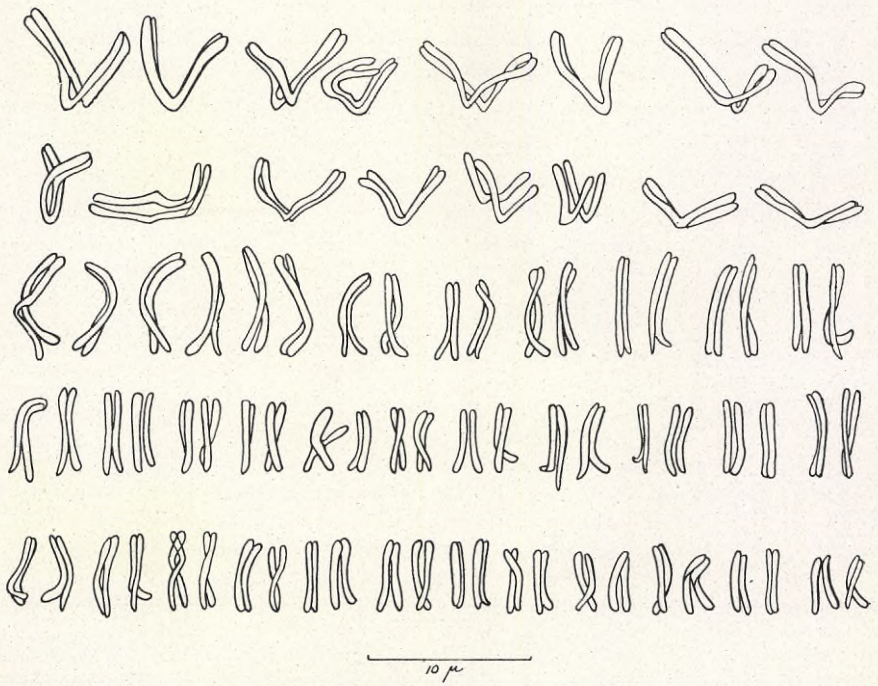


Fig. 4. Salmo alpinus. Mitotic chromosomes. Late metaphase, the centromeres have just divided (D).

Table 2. Means of chromosome length and breadth from table 1.

No.	A		B		C		D	
	length	breadth	length	breadth	length	breadth	length	breadth
1—16	10.16	0.465±0.013	11.70	0.743±0.005	8.69	0.681±0.011	8.96	0.451±0.011
17—32	7.73	0.429±0 013	8.03	0.725±0.015	5.89	0.658±0.011	6.21	0.403±0.007
33—48	5.95	0.468±0.014	6.39	0.688±0.014	4.75	0.653±0.014	4.98	0.395±0.001
49—64	5.44	0.463±0.011	5.54	0.696±0.012	4.45	0.654±0.013	4.47	0.401±0.009
65—80	4.65	0.430±0.013	4.81	0.728±0.013	3.95	0.675±0.013	3.99	0.408±0.007

1—80, according to the measured length. It is possible, and the present writer considers it even extremely probable, that *e. g.* No. 10 in a plate is not homologous with No. 10 in other plates, but there is no better possibility of homologizing the chromosomes in comparisons between different plates. For the present discussion, it does not matter at all if the chromosomes do not exactly homologize with one another.

In Table 1 the shortening percentages have been introduced with regard to the differences in chromosome length at different metaphases. Thus, on comparison between A and C, the difference in length for the respective chromosome has been computed in percentage of the original length, *i. e.* the length in A.

Text-figs. 1—4, in which each chromosome has been reproduced as drawn, and Table 1 show that the variations in size during the course of the metaphase are very considerable. The internuclear variation is likewise very great, and it should be noted that it is particularly large at this stage. After the lapse of 12 hours it has very considerably diminished.

In Table 2 the average length and breadth, and in Table 3 also the means of the different shortening percentages, have been introduced. If we first study the shortening from A to C, *i. e.* from pro- to mid-metaphase, it will

Table 3. Chromosome shortening.

Comparison between	Chromosome group					P
	No. 1—16	No. 17—32	No. 33—48	No. 49—64	No. 65—80	
A—C	14 5 %	22.4 %	20.1 %	18.2 %	14.9 %	$P < 0.001$
A—D	11.8 %	17.3 %	16.3 %	17.8 %	14.1 %	$0.01 > P > 0.001$
B—C	22.1 %	26.8 %	25.5 %	19.6 %	17.7 %	$P < 0.001$
B—D	19.6 %	22.2 %	22.0 %	19.2 %	16.8 %	$P < 0.001$

be found that the diminution in length varies between 14.5 % and 22.4 % for the different groups. Group 2, comprising the largest rod-shaped chromosomes show a higher shortening percentage and the other groups a lower, *the lower the shorter the chromosomes*. The difference between the various groups is considerable and the table also shows the value of P, *i. e.* the probability that chance alone has brought about the found distribution of the shortening percentages of the different groups. (The statistical methods are those of FISCHER, introduced in Sweden by BONNIER and TEDIN in 1940). As will be seen from the table, the value of P is less than 0.001, signifying that from a statistical point of view the differences found must be real and not due to chance.

Particularly interesting is the first group, comprising the V-shaped chromosomes. They show a shortening which is by no means in proportion to their total length, but instead entirely corresponds to the shortening in the smallest rod-shaped chromosomes. Table 2 also shows that the large V-shaped chromosomes have an average length of 10.16 microns, *i. e.* they have on an average arms of about 5 microns. These arms are thus quite as large as the smallest rod-shaped elements in the Salmo alpinus chromosome set. The conclusion must be that the shortening is not dependent on the total length of the chromosome, but, instead, on the length of the arm. *A large chromosome arm thus shows a relatively greater contraction than a smaller one.*

As previously mentioned, the late metaphase in D is not directly comparable with A or C, having too large chromosomes and at least one nuclear generation less behind it. No further shortening between C and D has therefore occurred, and the shortening between A and D will be less than between A and C. Besides the minor shortening, a comparison between A and D shows, however, the same relative changes in size as already noted, but there are certain minor deviations, owing to which the value of P rises over 0.001, but not over 0.01. The difference may thus be said to still be statistically significant. The explanation of this will be immediately obtained on a glance at the internuclear change represented by B and C. Here too the larger chromosome arms show a relatively greater contraction, which is statistically significant. Thus, if a prometaphase (A) is compared with a late metaphase (D) of a previous nuclear generation, the chromosomes of D, besides their greater length, must also show a less marked correlation between a high percentage of shortening and a great length of arm. The internuclear variation thus tends in this case to equalize the intranuclear change. From text-fig. 5, which illustrates schematically the found variations in size, it can be immediately understood why the comparison between A and D must show a somewhat worse correlation. If the late metaphase D, on the other hand, is

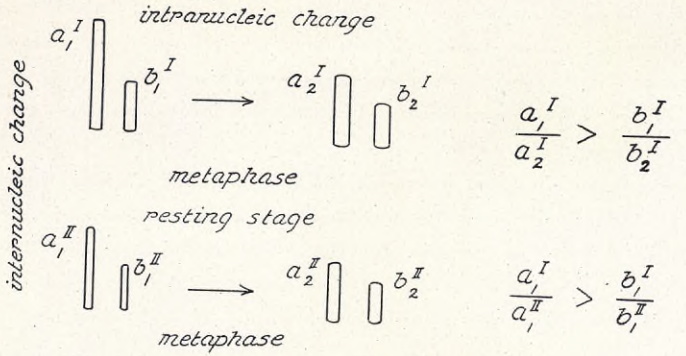


Fig. 5. Scheme of the chromosome volume changes of the Salmonoids.

compared with a mid-metaphase of the corresponding nuclear generation, *e. g.* B, the found correlation will appear again, with the usually marked statistical significance. However, the absolute differences in the shortening percentages this time are evidently less pronounced, which is interesting, as it suggests that the shortening is more marked between pro- and mid-metaphase than between mid- and late metaphase, which *a priori* must be designated as probable.

In Tables 1 and 2 the found chromosome breadths have also been included. In view of the fact that in such measurements the experimental errors must be considerably larger than in pure measurements of length, no real importance should be attached to the differences here found in regard to the breadth of the chromosome at different stages within the different groups. POWERS (1942) also found that the chromosome breadth could not be subjected to more exact measurements and conclusions. If the chromosome, for simplicity's sake, is regarded as a cylinder *(vide infra)*, and its volume is thus computed with the aid of the ascertained length and breadth measurements, we shall find that there is no simple connection between the shortening and the concurrently increasing, or decreasing, breadth.

From the above-mentioned analyses of the variation in size, it seems that the following conclusions can be drawn:

1. The length of the chromosomes diminishes both intranuclearly, during the course of the mitosis from prometaphase to late metaphase and also internuclearly, *i. e.* in the metaphases of successive mitoses, compared with one another.

2. This reduction in length is not proportionately the same for all chromosomes: on the contrary, decidedly *more marked in longer chromosome arms*

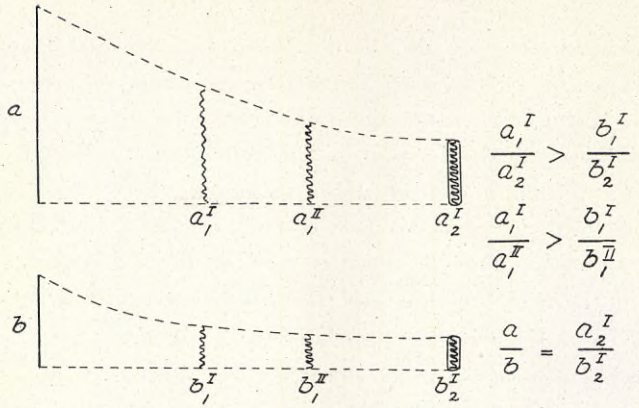


Fig. 6. Hypothetical explanation of the chromosome shortening during pro- and metaphase. The small chromosome (b) shortens more rapidly than the large one.

than in shorter, so that the chromosome set is subjected to a levellingdown in length.

3. If a chromosome has two arms, the contraction in both arms proceeds independently of one another, *i. e.* the contraction cannot pass the centromere. *Thus, if the two arms of the chromosome are of unequal length, the index showing the relative length of the arms will be modified.*

4. The measurements suggest that the said changes are most marked during the first part of the metaphase.

5. The breadth of the chromosomes does not stand, as regards volume, in any direct, simple connection with the shortening. During the course of the metaphase the breadth greatly increases, whereas during successive metaphases it decreases, despite the fact that in both cases the length of the chromosome diminishes.

Discussion. The possibilities of recognizing different chromosomes individually within a set from one cell to another show considerable variations. In many cases such homologizations have certainly been made on too loose bases, resulting in a maze of data regarding important chromosomes, such as the sex-chromosomes. WESTERGAARD (1940) thus referred the long-discussed problem of sex-chromosomes in *Melandrium* to such confusion, and he stresses the difficulties of recognizing certain definite chromosomes in different nuclei.

For a long time past it has been known that the absolute size of the chromosome cannot be taken as a characteristic. On the other hand, their relative size has been frequently adopted as a criterion (MC CLUNG 1905, MEEK 1913 and many subsequent authors). The first direct measurements that

were made in order to test this seem to have been those of POWERS (1942). He found that the relative size of the chromosomes was constant. On the other hand, LONGLEY (1941) questioned whether this relative size of the chromosomes was always constant, having found that the so-called B-chromosomes of *Zea* in somatic cells are about as long as the shortest other chromosomes, whilst in the prophase of meiosis they are smaller than half the smallest length of the others. This, however, in view of the inertness and the peculiar behaviour of the B-chromosomes (cf. the discussion regarding heterochromatin below, p. 36 and 41) may be a special case.

As regards two-armed chromosomes, an index has been used as a characteristic of the chromosome. In this index the length of the arms is indicated relatively to one another or to the total length of the chromosome. This method has sometimes been employed in such a way that considerable importance has been attached even to minute differences in the index obtained (*e. g.* EMSWELLER and JONES 1935). GEITLER (1938 a, p. 15) also states in his textbook: »Das äussere Aussehen des Chromosoms wird somit bei bestimmter Gesamtlänge durch das Längenverhältnis seiner beiden Arme bestimmt». Also subsequently (GEITLER 1940 a p. 649 and 1944) he maintained the same: »Durch das Centromer wird das Chromosom in zwei Arme oder Schenkel gegliedert, deren Längenverhältnis konstant ist». TISCHLER (1942) is of the same opinion: »Um ein gegebenes Chromosom eindeutig zu kennzeichnen, kann man nun den Quotienten nehmen, der aus der Division der Längenmasse für die beiden Schenkel oder Arme gewonnen wird» (p. 169). TSCHERMAK (1943, p. 511) who found in *Oedogonium* the ordinary chromosome contraction in colchicin treatment, says on this point: »Im allgemeinen werden alle Chromosomenabschnitte gleichmässig verändert. Nur schon im normalen Zustand besonders kurze Chromosomenschenkel können offenbar nicht so stark verkürzt werden wie die übrigen . . .» LONGLEY (1941) on the other hand, whilst pointing out the value of an arm-index in morphological studies of Zea chromosomes mentions that it is theoretically conceivable that »different sectors of the same chromosome do not contract simultaneously» (*op. cit.* p. 268). SINOTO (1938) and WOODS and BAMFORD (1938), who studied the characterization of certain plant-chromosomes by an arm-index, found that there was some variation in regard to the numerical value of the index. They did not make any real attempt at an analysis of this variation.

These rules, which have long been established, thus do not apply to the Salmonoids. Relative modifications, on the contrary are very marked here. On text-figs. 1—4 it will be seen for example at once that the longest rod-

shaped pair contracts much more markedly than the others. All the acertained differences in Table 3 are in fact statistically significant.

The natural question whether the Salmonoids are an isolated special case may be answered as follows. Within this group special conditions, particularly the protracted metaphase with a rapidly proceeding chromosome contraction, afford very favourable opportunities for studying the contraction. The chromosomes moreover are unusually well differentiated with large and small, two-armed and one-armed elements. Finally the number of chromosomes is so large that a statistical analysis can easily be made on homogeneous material. Owing to these factors, the relative changes in size can easily be determined, whereas in other material they are much less marked on account of the equal size of the chromosomes, or perhaps, owing to a shorter metaphase, take place only at the prophase, where measurements are always much more difficult to carry out. That the fundamental dissimilarity between long and short chromosomes has a rather wide, not to say general validity, seems therefore probable.

Possibly the phenomenon found in the Salmonoids may also give us some indications regarding the important problems of the chromosome structure and the cause of spiralization. The intranuclear variation in size as previously mentioned, is probably caused by a spiralization which from the outset of the prophase shortens and thickens the chromosome. Many studies on the detailed nature of the spiralization have been published, but the whole thing is still very diffuse and obscure.

A metaphase chromosome at mitosis consists of two chromatids, which are separately spiralized, but lie close to one another in a weak relational coiling (DARLINGTON 1937 and later, GEITLER 1938 a, 1940 a, 1943, and others). On the other hand STRAUB (1943), in a review of the chromosome structure, has recently contended that the two chromatids may also lie in a uniform spiral. His description however, is not clear on this point, as he shows pictures where the chromatids lie in different ways. GEITLER (1943) however, on the basis of favourable material, has actually been able to observe the flattening of the cylinder-shaped chromatids on the side where they lay close to one another. In the Salmonoids the chromatids certainly lie side by side, although they cannot be distinguished with certainty before the beginning of the anaphase. That they lie side by side and not in a common spiral is indicated by the fact that the chromosome bredth at late metaphase entirely corresponds to double the chromatid diameter. If they lay in a common spiral the diameter of the anaphase chromatids should be the same as that of the metaphase chromosome.

There is more divergence of opinion in regard to another very important factor, namely the question as to the hollowness of the chromatid spiral. WHITE (1940 b) considers that the spiral is completely closed, as also STRAUB (1943). DARLINGTON and UPCOTT (1939) on the other hand reckon theoretically with both kinds, close and hollow, respectively. That hollow spirals may occur is shown by the fact that GEITLER (1943) had actually observed them. »Überall wo ein Querschnitt optisch klar erfassbar ist, zeigt sich die Chromatide als Hohlcylinder» (*op. cit.* p. 527).

For all computation of the spiralization conditions, when one knows the length and breadth of a chromosome at two different times, it is necessary to know — in addition to the diameter of the spiral, the number of gyri and the possible hollowness of the spiral —, also whether the breadth of the chromosome (chromatid) really corresponds to the dimensions of the spiral. Here we are concerned thus with the very disputed term matrix. In latter years this term has been simplified in so far as it has begun to be viewed as a morphological description of the accumulation in the chromonema of nucleic acid in the course of the mitosis or meiosis (cf. CASPERSSON 1936, CASPERSSON and SCHULTZ 1938, CASPERSSON 1941). TISCHLER (1942) seems, however, to be of the older opinion. ELVERS (1943) has given a valuable literary review of the earlier reports regarding the matrix and spiralization etc., to which the reader is referred for details.

In this connection mention should be made of the view represented by ÖSTERGREN (1944 a). When the spindle breaks down owing to cold or colchicine or any other drug, the chromosomes contract with unusual intensity (DARLINGTON 1937, LEVAN 1938, TSCHERMAK 1943, BARBER and CALLAN 1943, LEVAN and ÖSTERGREN 1943, BÖÖK 1945, ÖSTERGREN 1944 a, and many others). It has been suggested that this is due to the fact that the chromosomes autonomously continue their spiralization, as it is not interrupted by any anaphase separation. ÖSTERGREN (1944 a) however is of a different opinion, and considers that the contraction is directly due to a change in the degree of folding of the polypeptide molecule chains. »In this way fibrous protein molecules are brought to change to a more or less corpuscular shape» (*op. cit.* p. 464). However, the facts he adduces in support of this view, in my opinion, can be completely explained by the assumption that the time-bound connection between the contractions of the chromosomes and the development of the spindle, or the setting-in of the anaphase, is disturbed in both directions. This, however, does not signify that his hypothesis need necessarily be incorrect.

Summing up, we must unfortunately note that our present knowledge of the chromosome structure at metaphase does not permit any deeper penetration into the purely physical nature of the spiralization. Nor can the measurements of the chromosome breadth given in Tables 1—2 be brought into reasonable connection with the changes in length. Of course, however, errors in measurements contribute to this uncertainty. So much, however, can be stated that the breadth of the studied metaphase chromosomes does not conflict with the assumption that at any rate the intranuclear variation is due to increased spiralization.

From theoretical points of view, it seems most reasonable that the length of the chromonema or of the gene-carrying protein thread should be constant. However, statements to the contrary are by no means lacking. WILSON and HUSKINS (1939) supposed that the increase of the chromonema in length in *Trillium* from diakinesis to anaphase is about double. COLEMAN and HILLARY (1941) confirm this observation. GRAFL (1940) considers that the varying chromosome size she has found in Oedogonium may be due to »verschiedene Wachstum der Grundelemente der Chromosomen» (*loc. cit.* p. 113). Also GEITLER (1940 a and b), as previously mentioned, has suggested this.

However, this possible capacity of the chromonema to contract without spiralization will not help to explain the changes in relative size which have been observed in the Salmonoids. If the protein thread is heterogenous in any respect, so that it can contract in a greater degree in certain parts than in others, it seems to the present writer inexplicable that this heterogeneity should be causally connected with the length of the chromosome arm.

The most reasonable explanation of the ascertained changes in intranuclear length should thus be sought in spiralization conditions.

It has long since been known that the pairing at pachytene and the chromosome division at diplotene move along the chromosome at a moderate speed. During the relatively short time which usually elapses between the beginning of the pairing and the setting-in of the diplotene division (growing oozytes, however, totally excepted) the chromosomes sometimes do not have time to pair in their entire length. There is thus a »time limit» for the pachytene pairing (DARLINGTON 1937 and later, BARBER 1942, WICKBOM 1945 and others).

Analogously, it might be supposed that the spiralization possibly »migrated» over the chromosome, with successive impulses from the centromere or telomere, in that case from both, as the spiralization can sometimes change its direction within an arm. In this way a shorter arm should thus have a lead in spiralization, which can be made good only gradually, at the end of the

spiralization phase, by the longer chromosome arm (see text-fig. 6). In that case the shorter chromosome arm during the prophase should shorten *more* and during the metaphase *less* than a long arm, as the analysis seems to show. A greater shortening of the smaller arm in prophase, however, cannot be shown in my material, as the chromosomes then cannot be closely analyzed.

This view as to the »migration» of spiralization along the chromosome should, however, entail the consequence that the ulterior cause of the spiralization or, at any rate, of the impulse to spiralization, should be sought next to the centromere and telomere. This is somewhat at variance with the hypotheses set up in order to explain the causes and nature of spiralization. According to DARLINGTON (1937), the causes of spiralization is to be sought in a molecular spiral within the protein thread. According to another hypothesis (KUWADA 1937, SAX and HUMPHREY 1939, WILSON and HUSKINS 1939) spiralization is produced by the fact that the chromonema increases in length, but is prevented by the matrix from expanding, being thus compelled to spiralize. According to a third hypothesis, finally (ELVERS 1943), spiralization is caused by an attraction and repulsion, respectively, of plus and minus poles in the nucleic acids lying across the chromosome axis.

However, it need not be at variance with DARLINGTON'S or ELVERS' hypotheses if the cause of the more rapid spiralization in a short chromosome arm is sought, instead, in the fact that this chromosome has a smaller *mass* than a long chromosome. If the molecules coil in a spiral and produce a torsion in the thread, which in turn induces a spiralization of the chromonema (cf. DARLINGTON 1937), a longer chromonema should possibly involve a greater resistance to the molecular torsion. The greater inertia of the larger mass should entail the result that somewhat longer time is required for spiralizing the longer chromosome arm in the same degree as the shorter.

This latter explanation seems simpler and for that reason is more credible. It can also receive support from a quite different quarter, namely from the development of chiasmata. According to DARLINGTON'S well-known hypothesis regarding the origin of a chiasma (summarized from his earlier work in DARLINGTON 1937) it can be said to be due to the torsion of the chromosome at the moment of dividing. As the new half-threads, which must be weaker than the original chromosome, are exposed to the torsion, one of them is broken, whereupon a series of events occur, which lead to the origin of a chiasma. Whether DARLINGTON'S hypothesis, as a whole, is correct or not, it is probable in all circumstances that torsion plays a large part (cf. DARLINGTON 1940 a). The chiasma frequency should therefore be affected by increased torsion. It is, however, known that the chiasma frequency is dependent on the arm length. From this rule, however, there are several

exceptions, one of which is of great interest in this connection, namely that »the shorter chromosomes usually have ... a higher chiasma frequency relative to their length» (DARLINGTON 1937, p. 144). So far as I know, this phenomenon has never yet received any rational mechanical explanation, but with the assumption that a spiralization (or torsion) sets in more rapidly in a smaller chromosome arm, owing to less inertia, *the higher chiasma frequency will be a natural consequence.*

As previously mentioned, the changes in length here discussed are fundamentally the same both for the intranuclear and for the internuclear variation. This seems to have certain consequences in regard to the possibility of theoretically explaining the internuclear variation in size. GEITLER (1941) has suggested three alternatives in explanation of internuclear variations in size: »1. Die Anzahl der (sichtbaren) Chromonemen wird vervielfacht; 2. Die Chromonemen setzen sich aus submikroskopischen Längselementen zusammen, die vervielfacht werden können; 3. Der chromonematische Aufbau der Chromosomen bleibt der Gleiche, es wird nur die Hüllsubstanz (Matrix) vermehrt ... — Die *Längen*zunahme der Chromosomen wäre in allen Fällen durch mässigere Spiralisierung zu erklären ... — Eine sichere Entscheidung zwischen den drei Möglichkeiten ist zur Zeit ausgeschlossen.» (*op. cit.* p. 40).

It seems probable to the present writer that the intranuclear increase in breadth up to the end of the metaphase must be mainly due to a greater accumulation of nucleic acid, as shown by CASPERSSON (cf. CASPERSSON 1941). In this way, GEITLER'S alternative No. 3 has gained in probability also in regard to the internuclear variation. *Thus, the internuclear variation in size can be explained by varying spiralization, accompanied by varying accumulation of nucleic acid on the chromosomes* (cf. KOSTOFF 1939 a, RESENDE 1940 and ELVERS 1943, p. 226, who have arrived at a similar conclusion from other starting-points).

This view is also strongly supported by the results arrived at by MAKINO (1941). In comparing the size of the spermatogonic chromosomes in *Mus caroli, molossinus* and *musculus,* he found that the chromosomes of *Mus caroli* were not only absolutely larger, but also showed the same relative conditions as the Salmonoid chromosomes (see MAKINO'S text-fig. 2 and his Table VIII, p. 334—335). He mentions it as specific of the species, without any attempt of explanation. If a greater and earlier terminated spiralization is assumed to occur in *Mus musculus,* we shall, however, obtain precisely the general levelling-down of the differences in size observed by Makino.

The considerable chromosome breadth in the first nuclear generations of the Salmonoids should, therefore, be connected with an increased amount of

nucleic acid in these nuclei. PAINTER (1940) has also pointed out that the need of nucleic acid is immense in the first stages of cleavage, and that in *Drosophila* such an accumulation is produced by the growth of the nutritional cells of the egg owing to endomitosis, so that they become highly polyploid, whereupon they are dissolved and the material from thousands of chromosomes is available in the egg plasma (PAINTER and REINDORP 1939).

2. The centromeres.

The position of the centromere is often marked by a constriction, termed by DARLINGTON (1937) primary or centric constriction, to distinguish it from other constrictions. On my aceto-carmine-fixed material there is no such primary constriction. To determine the position of the centromere is therefore by no means easy, *nota bene* if its position is to be ascertained exactly. In many cases I have therefore desisted from the attempt. Two-armed chromosomes, which occur in all the species examined and have also been reported by other investigators of Salmonoid chromosomes, have been determined in this respect on the basis of their situation at metaphase and the anaphase migration of the chromosome. The centromere was considered to lie at the apex formed by the chromosome at metaphase.

The great majority of chromosomes in the fish species examined are, however, »rod-shaped». As we know, the position of the centromere is now considered not to be quite terminal, with some special exceptions. (See the discussion on the origin of isochromosomes, etc. p. 105.) This rule, which has been ascribed to several different investigators, can in point of fact be traced back to AGAR (1913), who states (p. 292); »transverse segmentation of chromosomes is very widely distributed throughout the animal and vegetable kingdoms — probably, indeed, the potentiality to such segmentation is present in all chromosomes . . .»

Numerous »rod-shaped» chromosomes were in fact found, on closer investigation, to have an extremely small second arm. Such an arm was observed by PROKOFIEVA for example, in several organisms, *e. g.* precisely in salmons (PROKOFIEVA 1934), owing to the fact that the chromosomes were, as she puts it, »headed». In some chromosomes in *Salmo salar* such constrictions, however, could not be discovered, which she supposes to be due to the fact that the fixative had not penetrated with sufficient rapidity. This view of hers that the Salmonoids generally have two arms on all chromosomes has been accepted *e. g.* by GEITLER (1938 a).

I will not deny the possibility that PROKOFIEVA'S supposition may be correct, but, at any rate, in my preparations it could not be observed. On

the other hand, I have repeatedly observed how the spindle thread attaches itself precisely to the actual end of a »rod-shaped» chromosome. Recently HELWIG (1941) has again maintained that *Orthoptera* may have an abundance of true telocentric chromosomes.

In many cases it can be observed at metaphase that the actual tip of a rod-shaped chromosome is somewhat more lightly coloured and drawn-out. I consider it very probable that this represents a short heterochromatic segment near the centromere, such as has nowadays been found in many organisms and is supposed to be a regular phenomenon (cf. MULLER 1941 and others).

The division of the centromeres terminates the metaphase and introduces the anaphase. In all the Salmonoids examined there is a certain variation in regard to the time for the division of the centromeres. This is, perhaps, quite a natural phenomenon: especially in view of the large number of centromeres, some variation might in fact be expected. The found variation in regard to the moment when the centromeres divide can be observed in text-fig. 4 and in microphoto. 2.

Reports regarding the simultaneity in the division of the centromeres are scanty in the literature. UPCOTT (1939 p. 181) states, however, »In a normal mitosis the end of metaphase and the beginning of anaphase is marked by the division of the centromeres, which is simultaneous in all the chromosomes.»

It may be of some value to know the normal range of variation in regard to this cytological detail, seeing that a marked difference in regard to the beginning of the division is shown by certain genetic disturbances and heterochromatic fragment-chromosomes. A more or less pronounced lack of such simultaneity in the centromeres has been reported by HASEGAWA (1934), DARLINGTON (1936, 1937), UPCOTT (1937, 1939), BARBER (1940), DARLINGTON and UPCOTT (1941 a), and in all cases has been attributed to disturbances in the timing relationships between the chromosomes and the spindle. DARLINGTON and UPCOTT *(loc. cit.* p. 279) even consider that such phenomena can be attributed to »a weaker centromere, which although usually sufficient for its smaller size, sometimes fails to work in concert with the larger one». Thus, this weak centromere may divide either before or after the others.

In the Salmonoids, however, where the lack of simultaneity is not so extreme as in the other cases described, this seems to represent quite a normal condition.

3. Secondary constrictions.

Secondary constrictions are those which do not show the position of the centromere. They are of considerable importance, especially in view of the possibility they afford of morphologically characterizing a chromosome set. Their placing along the chromosome thread is also in fact mostly constant.

After HEITZ (1931 a, 1931 b) had discovered the connection between secondary constrictions and nucleoli, these chromosome parts have been studied with keen interest. The extensive literature which has since been produced on the subject has been summarized by GATES (1937), MENSINKAI (1939) and RESENDE (1940).

Various secondary constrictions without any connection with nucleoli have, however, also been found (see GEITLER 1938 a for the literature). This entire question, however, has been brought into a new light by DARLINGTON'S and LA COUR'S discovery of the allocycly of the heterochromatin (DARLINGTON and LA COUR 1938, 1940, 1941). Briefly, their discoveries show that numerous heterochromatic parts in the chromosome do not manifest themselves otherwise than possibly as chromocentres or prochromosomes (ROSENBERG 1909) in normal cases. If, on the other hand, the environment of the nucleus is changed, *e. g.* by cold treatment, these parts manifest themselves morphologically even on metaphase chromosomes, where they then appear as narrower and less stainable segments. The reason for their different morphological appearance is that in the resting stage they are overcharged with nucleic acid, whereas in cold treatment they are »starved» of nucleic acid.

The connection between such »differential segments» and the well-known nucleolar organizers (MC CLINTOCK 1934) is still very obscure. DARLINGTON (1941 a), in comparative studies of different *Paris*-species, has found indications that »the same genes which show allocycly in one species do not do so in another, *i. e.* allocycly and perhaps inertness are genotypically controlled, and that this control is related to the activity of the nucleolar organizers» (*op. cit.* p. 216). In another passage (*op. cit.* p. 206) it is stated: »This absence of allocyclic behaviour goes with the presence of nucleolar organizers. Within this group there may therefore be a correlation between the two, although in one species of *Fritillaria* allocycly and organizers are found together.»

On the other hand, organizers and allocycly have been found to exist together in a number of other organisms, *e. g. Adoxa* (GEITLER 1940 a), *Triton* (CALLAN 1942), *Triton, Bufo* and *Rana* (WICKBOM 1945), *Oedogonium* (TSCERMAK 1943).

In haploid rye LEVAN (1942) found a larger number of secondary constrictions than normally and states (p. 182)» ... in the haploid a certain degree of nucleic acid starvation seems to be the normal condition». This indicates that also genetic factors may produce this lack of nucleic acid in the heterochromatic chromosome sections. Similarly, KLINGSTEDT (1941) found uncoloured chromosome sections, interpreted by him as heterochromatic, in *Mecosthetus* in hybrids betweeen an English male and a Finnish female of this species. So far as is known, temperature conditions could not have played any part in this case, whence the development of these segments must probably be attributed to genetic disturbances resulting from hybridity.

Finally, WICKBOM (1945) has found that a shortage of nucleic acid is caused if the animals are exposed to starvation. Several different factors may thus apparently produce morphologically distinctive segments in cromosomes, where under normal conditions the heterochromatic segments — apart from the resting stage —, do not manifest themselves.

Here, however, it should also be mentioned that KOSTOFF (1938), by marked differentiation in staining, considers himself to have succeeded in showing »differential segments» in root-tip chromosomes of *Crepis,* where in ordinary staining no heterochromatic parts can be detected.

The heterochromatic segments hitherto found have had varying morphological development: in some cases distinct constrictions, in other cases parts with a smaller diameter than the rest of the chromosome, but not so thin as a normal constriction or Sat-filament. CALLAN (1942) found that at mitosis they have such a breadth that they must reasonably be expected to contain a spiral, whereas at meiosis they were thin as filaments and therefore presumably entirely unspiralized. CALLAN therefore supposes that the shortage of nucleic acid may be either partial or total and, if there is no nucleic acid attached to a heterochromatic segment, this segment cannot spiralize.

The Salmonoids show a large, but varying, number of constrictions. Firstly, there is a variation from species to species, in which respect the salmon (see below) has been found to have the most. In addition, however, there is a variation within different stages of mitosis, thus an intranuclear variation. Typical constrictions in *S. salar,* not primary, are shown in microphoto 1. Even in the previously studied metaphase plates of *Salmo alpinus,* text-figs. 1—4, the typical intranuclear variation of the constrictions can be observed. The prometaphase contains altogether 26 constrictions, the two mid-metaphases each 6, and the late metaphase plate none. The conditions are similar in all nuclei and in all species. *The number of constrictions is regularly highest at*

prophase, and afterwards rapidly decreases, so that at the beginning of the anaphase there is usually no typical constriction left.

Simultaneously with a decrease in the number of constrictions, they change character. At first narrow as filaments, they afterwards become thicker and stain like the other chromosome parts. As a rule the only way in which they can be recognized is that the chromosomes have a zone of weakness at the former constriction, so that they are liable to bend there. Especially at early metaphase, however, they are still distinctly somewhat narrower than the other chromosome parts. It seems probable, to judge by their morphological changes, that at early prophase they are unspiralized and more or less devoid of nucleic acid, whereas they afterwards spiralize and are charged with nucleic acid. Their behaviour very strongly indicates that — relatively to other parts —, they are *retarded in their mitotic cycle.*

KLINGSTEDT (1941) has thoroughly discussed the allocycly of the heterochromatin and shown that it is not only applicable to »a lower reactivity with nucleic acid» in the heterochromatin, as DARLINGTON and LA COUR (1940) considered. With reference to CASPERSSON'S results, he considers that allocycly can be better understood if the heterochromatin is regarded as retarded relatively to the euchromatin: »A displacement of the timing processes, in such a way that the heterochromites go through about the same cycle as the euchromites, but more or less out of step with the latter, would seem to account for all the known facts better» (*op. cit.* p. 172). This view of KLINGSTEDT thus receives strong support from the conditions in the salmons.

There are two types of secondary constrictions in the Salmonoids. The largest group shows the characters just described. The other group, comprising merely a few constrictions per nucleus, seem to have a greater tendency to persist to full metaphase and in fact do not disappear until just before the anaphase. These more stable constrictions can often be recognized from nucleus to nucleus, where they usually characterize some chromosome pair, which thanks to them can be homologized. This applies particularly to a chromosome pair with a long subterminal constriction (shown in text-fig. 1) in *Salmo alpinus* and a corresponding chromosome pair in *Salmo fontinalis* (see p. 68), where these constrictions are still more stable and are found at every metaphase.

The present writer presumes that these more stable constrictions are normal Sat-filaments. The nucleolus in these species is dissolved at a rather early stage of the prophase, whence no connection with certain chromosomes could be determined. On the other hand, in individual cases it has been observed that the nucleolus lies close to a constriction. Also at telophase it is unfor-

tunately impossible to ascertain what chromosome or chromosomes are concerned with the nucleolus. The number of nucleoli varies somewhat, being usually 1—3, thus approximately corresponding with the number of possible Sat-chromosomes. Theoretically, on the other hand, it is, of course, by no means inconceivable that all the constrictions observed in fact are concerned with nucleoli, *i. e.* are Sat-constrictions. In view of the large number and the fact that certain of them seem to be more stable, it seems, however, that the majority are so-called »Nicht-Sat-Differenzierungen» (cf. RESENDE 1940).

DARLINGTON and LA COUR (1940) found that the »differential segments» were constant in number, but of varying length, which they interpreted to signify that their *Trillium* specimens were hybrids. This would afford very valuable facilities for studying the frequency of structural differences in normal populations. KLINGSTEDT (1941) actually states ... »the comparative study of the distribution of eu- and heterochromatin ... will open up new opportunities, which will somewhat make up for the great advantages the salivary gland method has given to *Diptera* in comparison with other groups» (*op. cit.* p. 171).

CALLAN (1942) on the other hand, who obtained also in cold treatment a constant number of segments from cell to cell, considered that he could not determine with certainty any hybridity, which, however, can of course, be explained by the fact that the animals examined were entirely structural homozygous.

In the Salmonoids this method is, unfortunately, completely ruled out, as the number and form of the constrictions vary from nucleus to nucleus. Chromosomes which for other reasons must be considered to be homologous sometimes show a constriction at the same place, sometimes at different places, sometimes merely in one of the chromosomes or, perhaps, in another nucleus at the same mitosis stage, no constriction at all. The occurrence of constrictions in these animals thus seems to be a characteristic of the nucleus and not of the individual. This variation seems to indicate that the form of the constriction is dependent on the local supply of nucleic acid within the nucleus, so that, in some measure there is a »competition» between different heterochromatic segments in regard to nucleic acid. Great caution must thus be observed in judging the real structure of the chromosomes on the basis of the constrictions which they show.

If such differences may exist within a single nucleus, it seems conceivable that the differences found by DARLINGTON and LA COUR in the length of the heterochromatic segments need not be attributed at all to hybridity. It is, however, possible that the lack of nucleic acid in the Salmonoids is merely slight, whereas it is considerably more marked in cold treatment, as

performed by DARLINGTON and LA COUR as well as CALLAN. In both these cases the number of segments was at any rate stable, or at least more stable than in the salmons examined. In order to test this hypothesis, cold experiments were made, but so far they have not given the result that a significantly higher number of constrictions had been formed. Nor were they found to be more constant, but varied as in normal temperature.

No special reason for the deficiency of nucleic acid in the Salmonoid embryos has been discovered. The phenomenon, however, is most marked in the large early nuclei, where the need of nuclei acid (cf. PAINTER 1940 and the discussion p. 32) should be high. In this case we thus seem to be concerned with perfectly normal conditions.

4. Orientation in the metaphase plate.

In somatic metaphases the chromosomes as a rule arrange themselves in such a way that the larger elements lie along the periphery of the plate, the smaller, on the other hand, further in towards the centre of the plate. The orientation for each chromosome is normally autonomous, *i. e.* only its centromere and the »body repulsion» (DARLINGTON 1937) determine the position of the chromosome in the plate, and different chromosomes lie distributed at random within the available space. From this rule, however, there are exceptions, where also some other force seems to be influencing the position of the chromosomes; as a rule the homologous chromosomes then lie closer to one another than could be attributed to mere chance. This phenomenon, which DARLINGTON terms somatic pairing, is best known from *Diptera,* but is also found among many other organisms, both plants and animals. Its occurrence is discussed at some length by TISCHLER (1942) who comes to the conclusion that in most cases it is solely due to a quite random arrangement of elements of fairly equal size.

In a previous paper (SVÄRDSON 1941) I mentioned somatic pairing in the Salmonoids and also hypothetically tried to explain it as a consequence of attraction between the homologous chromosomes at anaphase. The hypothesis is based on DARLINGTON'S view that the anaphase chromosome is simple and also on his theory of pairing between twos, but was extended to the whole mitosis.

New evidence in support of my hypothesis has scarcely been produced since then. On the other hand, it has been somewhat weakened by the following two new facts: 1. The difficulties of homologizing two chromosomes with certainty have considerably increased, which is a consequence of the ascertained variations in size and the shifting appearance of the constrictions.

Figs. 7—9. Salmo alpinus. Mitotic metaphase. The chromosomes, showing an end-to-end orientation are defined by thicker lines.
Figs. 10—11. Coregonus lavaretus. Non-disjunction.

2. The observed occurrence of two quite similar chromosomes in the microscope, sometimes lying close to one another at anaphase, has been shown — at any rate in certain cases —, to be certainly due to non-disjunction.

The metaphase orientation, however, has been further complicated by a new factor, a new disturbance of »normal» conditions. This disturbance was discovered in *Salmo alpinus*, where it is much more marked than in other

species, owing to the fact that the chromosome number, on a rapid estimate, was too low and the number of V-shaped chromosomes consequently too high. Good plates had previously shown the normal number of chromosomes. A closer examination of the apparently deviating metaphase plates revealed, however, that the supernumerary V-shaped chromosomes in fact consisted of two rod-shaped chromosomes, lying close to one another, with their centromeres close together. Also larger groups of three or more chromosomes could be found lying with their proximal or distal ends close to one another (see text-figs. 7—9).

These positions in the metaphase plate could scarcely be due to chance, and to the effect of the smearing — although it must be admitted that the rôle played by chance in such cases is difficult to compute mathematically and thus still more difficult merely to estimate —, seeing that three »subspecies» of char, examined in different years, all showed exactly the same phenomenon. The tendency to such an end-to-end orientation, however, greatly varies from plate to plate.

Various cytological observations have been declared by the discoverer or others to be artefacts produced in or as a result of the fixation, and therefore by no means typical of the living nucleus. DARLINGTON (1937, p. 565), however, says some sensible words on the subject: »No appearance of treated material is definitely free from artefacts, nor is any appearance 'pure'artefact. The question is therefore not so much whether an appearance is an artefact, but how significant the artefact is.»

The observed phenomenon has various points of contact with »clumping», due to bad fixation. Even if this should be the explanation, it seems to me to imply that there are certain important differences in »clumping capacity» between chromosome ends and other chromosome parts, which is of interest.

There are in fact other indications which point to the existence of a more or less fundamental difference between chromosome ends, centric regions and other parts. DARLINGTON (1937) pointed out that proximal and distal parts show in principle a marked tendency to become inert or heterochromatic. The more recently studied cases of nucleic acid starvation (DARLINGTON and LA COUR 1940, 1941, CALLAN 1942, LEVAN 1942, WICKBOM 1945 etc.) have shown that this assumption has been confirmed. Thus (cf. MULLER 1941) it is justifiable to suppose that chromosome ends and centric parts are often heterochromatic, though, on the basis of a certain material, there is still no direct evidence of this. The difference between eu- and heterochromatin in regard to spiralization and nucleic acid attachment has been previously discussed.

There are also other differences of this nature. If a chromosome is broken

spontaneously or owing to a certain treatment, the broken parts have a strong tendency to reunite. On the other hand, it seems that as a rule no reunion takes place between the fractured surfaces and chromosome ends (cf. MULLER 1941, who discusses this matter in detail). The distal part has therefore also been called telomere, in order to mark its deviating behaviour from that of the other chromosome parts.

In *Zea mays,* where the distal parts are certainly heterochromatic — a review of the chromosome morphology of the species has been given by LONGLEY in 1941 —, BEADLE (1932, 1937) showed a gene which produced »stickiness», that is a tendency to the sticking together of the heterochromatic parts. The phenomenon was particularly distinct at anaphase. The same result can be obtained in *Allium* with the aid of certain chemicals (ÖSTERGREN 1944 b). In cold treatment the »stickiness» is common, though DARLINGTON and LA COUR (1940) at first interpreted it as an absence of chromatic division within this region. Afterwards (DARLINGTON and UPCOTT, 1941 b) it was supposed that delayed reunion (from previous breaks) existed, so that reunion in heterochromatic parts would be caused by the cold treatment.

Additional examples of the »differential behaviour» of the heterochromatin may be adduced. RIBBANDS (1941) found in *Diptera* an attraction between non-homologous chromosomes, which, after discussion, he attributed to special properties in the chromosome ends, centromeres and centrosomes. RIBBANDS went through the rather extensive literature regarding such non-homologous attraction, which, however, in no previous case appears to have been observed in the mitotic metaphase as in the char. Most investigators seem inclined to attribute this attraction to the occurrence of heterochromatin, a view which RIBBANDS refuses to endorse. On the other hand, SCHRADER (1941), after describing some additional new cases of non-homologous association, accompanied by heteropycnosis, rejected RIBBANDS' view.

TISCHLER (1942, p. 181) reports the observations made by GEITLER and others in studies of endomitosis, in which, as we know, the homologous chromosomes lie near one another after polyploidization. The heterochromatic chromosomes then show a very strong affinity to one another.

Finally, we may refer to the known occurrence in the nuclei of the salivary glands of chromocentres forming a connected structure including all the heterochromatic parts in the different chromosomes (literature in RIBBANDS 1941).

Summing up, we must admit that there are various indications all of which seem to signify that the heterochromatin under certain conditions has a greater affinity, or less repulsion, than the euchromatin. Under such conditions

the end-to-end orientation in the mitotic metaphase, which has been observed in *Salmo alpinus* as well as in other Salmonoids, must probably be assigned some significance. This, of course, does not mean that such orientation occurs in the metaphase of the living nucleus. On the contrary, it is quite possible that the orientation is produced in the fixing, owing to the fact that the eu- and heterochromatin then react differently.

5. Non-disjunction.

Ever since BRIDGES (1914) the term non-disjunction has been used to designate the fact that two chromosomes or chromatids are not normally separated at anaphase, but for some reason passed in the same direction. DARLINGTON (1937, p. 579) however, limits the use of the term merely to meiosis. In the sequel, however, »non-disjunction» will be used also in regard to corresponding disturbances in mitosis.

At an early stage of my cytological studies on the Salmonoids I had already suspected that the chromosome number in exceptional cases was not the normal diploid number, but in certain cells exceeded or was less than that number. Various metaphase plates thus showed an odd number of chromosomes. But the difficulties of determining non-disjunction with certainty are great, especially as with the smear method it may naturally happen that the metaphase plates are spread out so much that some chromosomes loses contact with the others and in the preparation cannot be found in the vicinity of the metaphase plate. Moreover, the constrictions may sometimes be so thin or possibly broken, that it is practically impossible to decide whether one or two chromosomes are present.

In *Coregonus lavaretus,* however, various disturbances occur in the normal course of the mitosis (*vide infra* p. 72). In regard to this species it could be determined with certainty that the non-disjunction occurred, and indeed on a rather large scale. Although in other species non-disjunction has never been directly observed, I consider it therefore probable that it does occur, though doubtless on a smaller scale than in gwyniad.

Twenty extremely distinct metaphase plates of a series of simultaneously fertilized gwyniad embryos showed the following chromosome numbers:

78	79	80	81	of which in the same	78	79	80	81
2	3	11	4	embryo	—	1	6	4

In *Coregonus lavaretus* there also occurs an extremely small chromosome-fragment — which shows marked non-disjunction. The presence or absence of the fragment, however, has not alone entailed the above variation in the

chromosome number. This is indicated by the fact that the morphology in the metaphases examined varied in such a way that sometimes a V was missing, and that sometimes supernumerary V's were found, and so on. Also among the 11 metaphases, containing exactly 80 chromosomes — which is the normal number —, variation occured in the chromosome morphology. That this variation was due to non-disjunction was shown by several direct observations at anaphase (see text-fig. 10—11 and microphoto. 32).

The course of events in non-disjunction is the following. For some reason a chromosome remains lying in the metaphase plate with a (probably) undivided centromere after all the other free chromatids have begun their anaphase migration. The centromere may be called »weak», as it is not merely in regard to time of division that it deviates from the others. Also the repulsion between the two daughter-centromeres is distinctly less than normal, possibly even non-existent, as the free chromatids lie some distance from one another, but almost parallel. In DARLINGTON's terminology, the repulsion may have been caused solely by the »body repulsion» of the chromatids.

DARLINGTON (1937) has suggested that a normal anaphase is produced by two fundamentally different forces. On the division of the centromere a repulsion between the daughter-centromeres immediately sets in. This force draws the chromatids from one another, being strengthened by their body repulsion. When this first *centromere spindle* has been formed and separated the cromatids, they are carried further from one another owing to the fact that the *centrosome spindle* passes through a stretching which begins in the central parts, *i. e.* in the plane of the former metaphase plate. These hypotheses of DARLINGTON correspond well with the course of non-disjunction in *Coregonus*.

The belatedly free chromatids in fact continue here to lie in the plane of the former metaphase until they are shifted — very slowly — somewhat towards one of the poles, but still lie rather close to one another (text-fig. 11). This latter migration should therefore be attributed to the stretching of the centrosome spindle.

The line of demarcation between non-disjunction and chromosome elimination is vague. The only difference is that in the latter case the migration is so slow that the chromatids, which sometimes may also continue to hang together in an undivided centromere, are not incorporated with either of the daughter-nuclei, but remain lying outside. An undoubted elimination, however, has seldom been observed in any of the pure species examined, but occurs in hybridization or genetic disturbances of an unknown nature (see p. 62 and p. 73).

Non-disjunction in somatic tissues does not seem to be so rare, although,

so far as I am aware, direct observations are not available. The chromosome number, however, according to very scattered reports in the literature, now and then shows a variation which is usually attributed to presumed non-disjunction. That other and more marked disturbances may nevertheless occur and account for these deviating chromosome numbers seems, however, possible and will be exemplified below also in regard to the salmons.

B. The special morphology of Salmonoid chromosomes.

One of the principal objects of this study on the cytology of the salmons was to determine the exact chromosome morphology for as many species and subspecies as possible. This is particularly important, since these salmons have been split up into a number of populations, separated from one another by impassable land areas, during which time systematic differentiation in many cases had made some advance, despite the comparatively recent date of the isolation. The evolution of fishes moreover shows various special features, which will be dealt with elsewhere.

Thanks to the fact that the Quartenary Geology of Sweden is so well known, the age of the populations in most cases can be determined with considerable exactitude, so that interesting conclusions regarding the constancy of the chromosome morphology can be obtained. Examples of this will be given in the sequel.

Under the respective species, the meiosis, where studied, is also dealt with, whence the term »morphology» should be understood in its widest sense.

1. The Salmon (Salmo salar L).

Mitosis. The chromosomes of the salmon have previously been studied only once, namely by PROKOFIEVA (1934 a and b). She states that she had obtained the best pictures in embryos aged 6—8 days (at what temperature the development had proceeded is not mentioned). Younger embryos indeed have larger nuclei, but the number of mitoses here is too small. After the lapse of 8 days the nuclei, according to PROKOFIEVA, have become so small that it is very difficult even to determine the number of chromosomes, whence it is quite impossible to study their morphology.

Prokofieva determined the diploid chromosome number at 60 and, in respect of morphology, she divided them into several groups: »long equilateral (4 pairs), long, inequilateral (2 pairs), long, headed and short, headed» (being

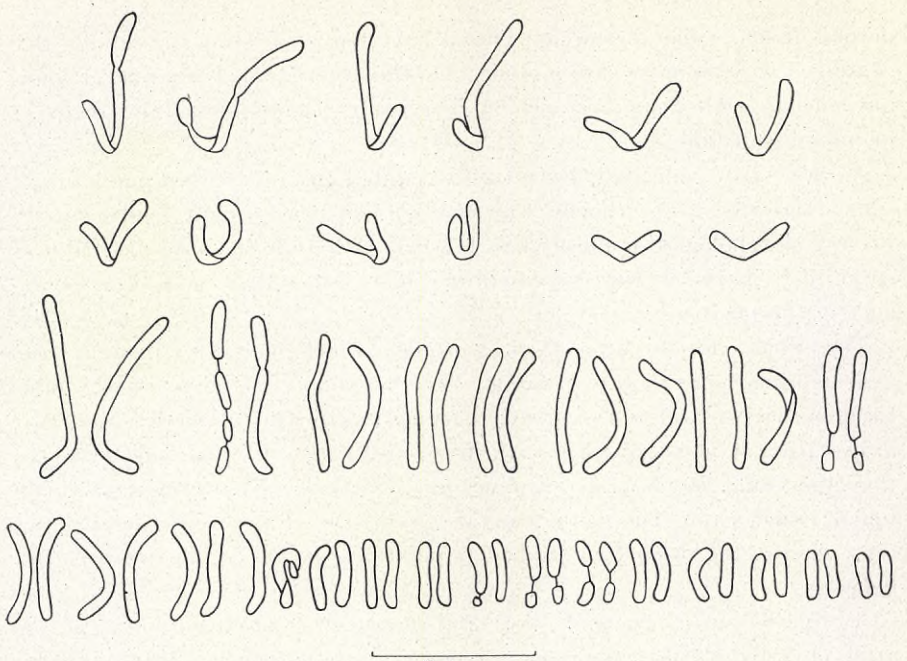


Fig. 12. Salmo salar. Mitotic chromosomes.

the bulk of them). In addition, she found also a pair of chromosomes with satellites (trabants).

My own material of *Salmo salar* is derived from three different localities. Thus one sample is from the salmon in the Baltic, more strictly from the population that spawns in the River Dalälven (from Älvkarleby Salmon Breeding Institute), another from the west coast of Sweden, namely from Falkenberg, where the salmon of the Kattegatt ascend the river Ätran, to spawn. Finally, the third sample comes from Karlstad in Värmland, where the landlocked salmon in the lake Vänern ascend the river Klarälven, in the spawning season.

Good pictures have been obtained of all the samples, after fixing and dyeing. It was found necessary, however, to use considerably younger stages than those studied by PROKOFIEVA, namely embryos aged 3 days after fertilization (temperature 8—10 centigrade).

PROKOFIEVA'S preparations must have been better than is indicated by her published pictures. The number given by her, $2n = 60$, is undoubtedly correct, and her morphology is in fairly good correspondence with my results (text-fig. 12 and microphoto. 3).

As previously mentioned, nuclei with a higher or lower number than the

normal diploid sometimes occur. Thus I have found nuclei in the salmon that contained only 59 or 58 chromosomes. On the other hand, I have never found any nucleus with a number over 60. The cause of these deviations seems to be non-disjunction.

As previously indicated, PROKOFIEVA stated that all the salmon chromosomes had two arms, though the smallest arm was usually quite minute. From a morphological point of view, however, I consider it quite justifiable to speak of V-shaped or rod-shaped chromosomes, since they have that appearance in the microscope.

The salmon has 6 pairs of chromosomes, which have two distinct arms and are thus more or less V-shaped. The remaining 24 pairs, on the other hand, are rod-shaped and of greatly varying length. Out of the 6 larger, two-armed pairs, 4 pairs have arms which are distinctly of equal length and are thus the »long, equilateral» chromosomes mentioned by PROKOFIEVA. She found 4 such pairs. This is the case also with the salmon from Älvkarleby, whereas the other two studied populations deviate in this respect (see below).

A division into long and short rod-shaped chromosomes, such as was attempted by PROKOFIEVA, cannot be carried out, seeing that the arm-length within this chromosome group forms a slowly and evenly falling curve.

The number of secondary constrictions in the salmon is large. As in all the other species, the constrictions vary greatly from nucleus to nucleus and from prophase to anaphase. One chromosome pair had constrictions developed in all the thoroughly examined metaphases. It is one of the very smallest pairs of rod-shaped chromosomes, in which the constriction is situated exactly in the median line. Very often, in another pair, there are constrictions of somewhat greater length, though they are not situated medianly, but more proximally. In about the 10th pair, reckoned from the smallest rod-shaped, there is also a proximal constriction, which rather often is fully developed. It is possibly this chromosome pair to which PROKOFIEVA is referring when she speaks of a chromosome with a satellite. Besides these more stable constrictions, there are a number of others, greatly varying. The largest chromosome pair — J-shaped — often has a constriction in the middle of its long arm. Also the next longest rod-shaped pair often has one or more constrictions. Finally, many of the rod-shaped chromosomes round the proximal end sometimes assume an appearance which distinctly indicates that there are heterochromatic parts there. The end seems to be slightly pointed, and sometimes lighter in colour. This is presumably the explanation of PROKOFIEVA'S »headed» chromosomes. She in fact admits that she had not found all chromosomes provided with such a, supposed primary, constriction. As previously

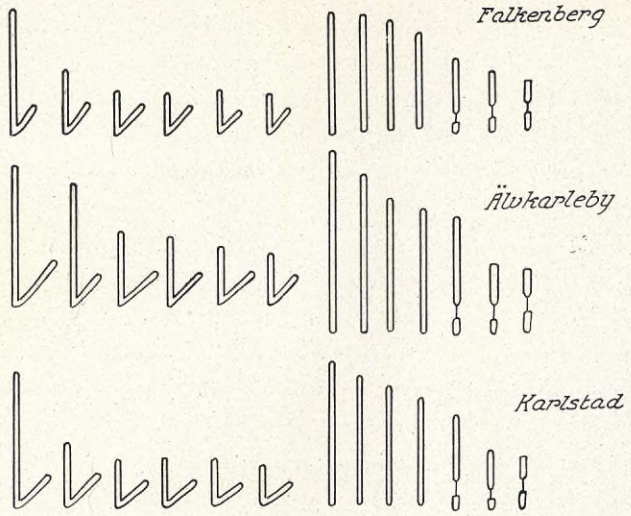


Fig. 13. Salmo salar. The chromosome morphology of three different populations. Note: the second large J-shaped chromosome of the Baltic population (from Älvkarleby).

Fig. 14. Salmo salar. M I showing 30 bivalents.

Fig. 15. Salmo salar. M I showing univalents.

pointed out in the general survey, the centromere was never observed as a constriction in the material fixed and stained by me in acetocarmine.

PROKOFIEVA, unfortunately, did not state from what locality her material was derived. But, as her investigation was made at Leningrad (PROKOFIEVA 1934 b), the material appears to have come from the Gulf of Finland, *i. e.* from the Baltic. This is a matter of some importance, as it has been found that the three populations studied by me differ in respect to one of the large J-shaped chromosome pairs. Prokofieva found two such pairs. This is the case also in my material from Älvkarleby (also from the Baltic), whereas the salmon from Falkenberg and from the lake Vänern show only one such J-shaped pair and, instead, have five V-shaped chromosome pairs among the large two-armed chromosomes. The

differences between the chromosome sets have been illustrated in text-fig. 13. I hope later to be able to illustrate this difference with more material from other localities. The four studied populations, however, seem to give some support to the view that *the Baltic salmon population has a deviating karyotype, as compared with the salmon off the west coast of Sweden and in Lake Väner.*

We know, by marking of salmon, that these fish as a rule return to the river in which they had been hatched, although before their sexual maturity (some males, however, become sexually mature even as young) they roam about in the sea within an extensive area. For the spread of the species, however, it is, of course, necessary that the salmon should spawn also in other places than those in which they had been hatched.

As to the time when the salmon migrated into this country we have no certain knowledge, except that this must have happened after the last glacial period. Possibly the salmon had already occurred in the glacial sea which arose when the great inland ice began to melt. During this period arctic flora and fauna migrated into the south parts of the country, and we also know that man existed towards the end of this epoch. The large Baltic glacial sea was not in open communication with the Atlantic, but debouched by river through the present Sound into the Skagerrak. If the salmon occurred in this sea — which in view of the present distribution of the species, is quite conceivable —, it may have entered it *from the east,* since the glacial sea — at any rate temporarily — was in communication with the White Sea (Ekman 1922, Magnusson and Granlund 1936). Subsequently, as we know, the sea extended across central Sweden, thus affording facilities for communication between the salmon populations in the Baltic and off the west coast.

It is, however, just conceivable that the chromosomal difference between the Baltic and west coast salmon populations is due to a double migration into this country of salmon both from the east and from the west. It is also possible, however, that this difference, which need not mean more than an inversion, comprising the centromere, may have arisen far later.

This difference is of great interest and the resemblance between the chromosomes of the salmon in Lake Väner and the salmon off the west coast is also significant. As regards the salmon of Lake Väner, it can in fact be stated that towards the end of the A n c y l u s-period it had become isolated in that lake. Thus, despite the fact that the two western populations had been isolated from one another for roughly 8 000 years, no morphological differences could be shown in the chromosomes. During this period the populations

seem indeed to have been differentiated from one another genetically, so that they now show certain morphological differences, but investigations which would enable us to determine with certainty whether these differences are partial modifications or not, are not as yet available.

Meiosis. The meiosis of this species has not previously been studied. My material is derived from the fishery experimental station at K ä l a r n e in J ä m t l a n d, where salmon fry from the Baltic population are reared in ponds. The males usually become sexually mature at the age of two to three years, *i. e.* before the normal migration into the sea, as is now a well-known fact, but the females seldom become sexually mature in ponds and, if so, only after reaching a high age. The meiosis takes place in the late summer; my material is from the month of August and comprises three specimens.

The meiosis of the salmons is extremely difficult to study, owing to the very large number of chromosomes and the minuteness of the nuclei. Acetocarmine gave the best pictures (text-fig. 14 and microphoto. 4—6). It is very characteristic of the meiosis of the salmon that, in many cases, the metaphase of the first meiotic division (M I) is disturbed by the occurrence of univalents lying outside the metaphase plane. It is impossible to give an exact figure for the frequency of disturbed divisions, but they may be roughly estimated at 10—20 per cent.

In text-fig. 15 some of such disturbed M I are shown from from the side. Besides the univalents, one can sometimes see pictures which can scarcely be interpreted otherwise than as »chains», that is, in addition to univalents, also multivalents must occur. Despite much labour, merely 5 metaphases have been completely analyzed.

	I	II	III	2 n
3 nuclei	—	30	—	60
2 »	1	28	1	60

Microphotograph 4 shows a normal M I, without disturbances. The large bivalents are naturally the large V-shaped chromosomes. In cases where the bivalent could be closely examined from the side, it has always been found that the chiasmata were situated quite terminally.

As also other species show meiotic disturbances of a similar nature, they will be discussed in their entirety further on, see the Chapter »Meiotic Disturbances», page 109.

2. The Brown Trout (Salmo trutta L).

Taxonomy. In the salmon family there are some species which present the greatest difficulties to the taxonomist. This applies particularly to the gwyniad and the brown trout. Here I shall merely briefly point out the errors in regard to the systematic view of the different forms of the trouts which are very common to this day.

The trout spawns in fresh running water. The young remain in this running water for a shorter or longer time. If the running water opens into the sea, the young as a rule gradually migrate into it, grow there very rapidly, being then bright in colour, and return after a few years to the running water, where they assume a so-called spawning dress in gay colours, and spawn. The life cycle of these salmon-trout is thus in all essentials similar to that of the salmon. If the running water opens into a lake, the young migrate into it, grow there and then return to the brook. In this case their growth is not very rapid, but they are nevertheless bright in colour before they assume their spawning dress. In small running waters, on the other hand, which do not soon open out into any lake, the trouts may remain for their whole life. They are then of stunted growth, never assume the bright dress and become sexually mature after a rather short space of time, sometimes three years. Sexual maturity may thus set in at any time during a sequence of years and is undoubtedly influenced by the environment (cf. Svärdson 1943).

These three different types of brown trout are usually designated by subspecific names and are termed respectively *Salmo trutta trutta, Salmo trutta lacustris* and *Salmo trutta fario*. (Neresheimer 1937 calls them also species.) Apart from the fact that modern investigations (Alm 1939, Steinmann 1941, etc.) have shown that these different types are chiefly modifications, nevertheless, even if these distinctive characters in regard to habit of life and morphology were genetically determined, the principle of regarding these types as three different subspecies must be incorrect. There is in fact no reason whatever to believe that these three types, which are now found spread mosaic-like over the country and are naturally connected with all kinds of conceivable transitions, are of monophyletic origin, so that for example all *fario* types would be derived from a *fario* type which had migrated into the country. On the contrary, they have in all probability developed independently of one another within isolated lake areas owing to parallel adaptation to similar environments. In regard to other characters, they are in all probability different from one another, seeing that they have been isolated from each other for a very considerable space of time, especially as the populations were quite small. This phenomenon is interesting and hitherto, so far as I

can find, unnoticed despite the fact that intense study has been devoted to the systematics of these fishes. A direct parallel to this is afforded by the so-called gwyniad species introduced by THIENEMANN and others, where the convergent development comprised the formation of gill rakers, whereas a number of other characters »vary» within »the same species» from locality to locality and are therefore not assigned »systematic value» (see *e. g.* THIENEMANN 1928). These apparent species or subspecies, which may be set up when the systematic examination is confined merely to such a character as shows marked convergent adaptation to a certain environment, are evidently partly of the types which TURESSON (1922, 1925, 1931) has called ecophenes and otherwise »ecotypes in being». I intend to deal with these questions more thoroughly in another connection, but I have considered it necessary briefly to mention them here, in order that no doubt need arise as to which form has been cytologically studied.

Mitosis. All the three above-named »subspecies» of brown trout in this country have been cytologically studied. My material of *fario* is derived from K ä l a r n e in J ä m t l a n d, *lacustris* has been obtained from K ä l a r n e and Lake Vätter and the »subspecies» *trutta* from Ä l v k a r l e b y.

PROKOFIEVA (1934) had most roe of this species, so that she could try several different kinds of fixatives. Nevertheless she was obliged to admit that »the chromosomes displayed a tendency to stick to one another under every fixative tested» (p. 503). I have, in some measure, had the same experience. Despite the fact that the same acetocarmine method was employed in regard to all the species, the metaphases of this species were by no means easy to study. Moreover, I noticed that the spindle evidently showed greater stiffness in this species and did not easily permit the chromosomes to spread radially outwards on pressure in the longitudinal direction of the spindle. It seems therefore quite conceivable that the consistency of the spindle in the brown trout is in some respect different from that of the other species. This matter, however, has not been more closely investigated, being beyond the scope of this study.

After repeated fixings during each season with gradually improving results, I could, however, state with certainty that the number of chromosomes in the brown trout is diploid 80, and not 84, as contended by PROKOFIEVA. All the four populations studied by me, representing all the »subspecies» in the country, have $2n = 80$ (microphoto. 7), whence PROKOFIEVA'S number is certainly incorrect. She may possibly have happened to mix up the roes, as she also gives the number 80 for *Salmo fontinalis*, which instead has 84 *(vide infra)*. However, it is quite possible that she had simply mis-

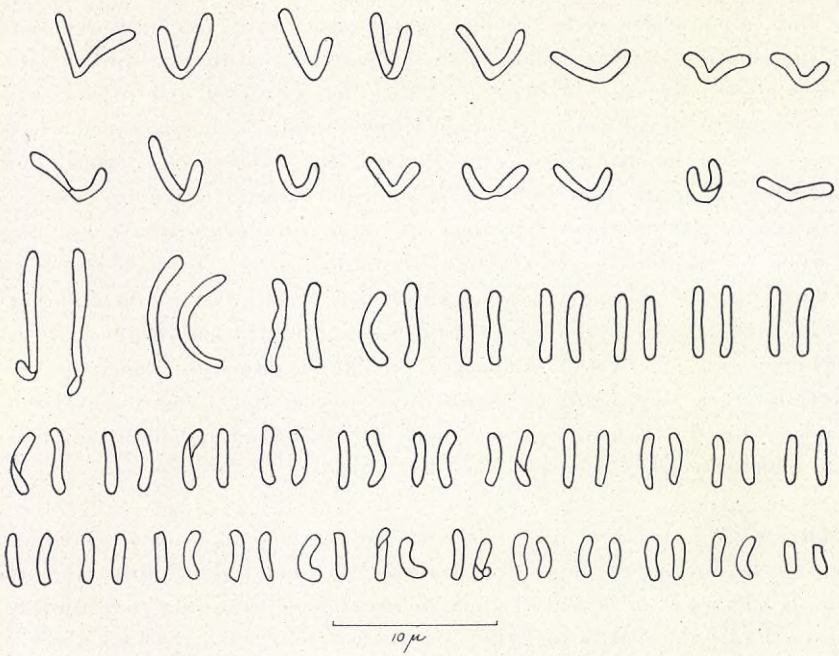


Fig. 16. Salmo trutta. Mitotic chromosomes.

interpreted her metaphase pictures, as they were not well fixed. This is borne out by the fact that POMINI (1939), who also had bad pictures and I myself, in a preliminary report (SVÄRDSON 1941) had been influenced by PROKOFIEVA'S pictures to interpret the chromosome number of the brown trout as the number stated by PROKOFIEVA.

PROKOFIEVA, owing to her unsatisfactory pictures, could not state the frequency of the long, equilateral and the long, inequilateral chromosomes, as she calls those which are V- and J-shaped. In my material, however, apart from the inferior pictures of the first years, I have constantly found 8 pairs of V-shaped chromosomes (text-fig. 16). In 6 of these chromosome pairs the arms are fairly equilateral, whereas in one pair they are somewhat different in length and in another pair markedly different, the short arm being only about half the length of the longer one. No certain differences as regards the relative length of the arms have been found in the different populations, despite the fact that they have been isolated from one another for many thousands of years.

In addition to these 8 pairs of clearly V-shaped chromosomes, there is also another pair which is two-armed, although the short arm is very small. Moreover, this short arm is heterochromatic, at any rate in part, which is

indicated by the fact that it sometimes develops constrictions. Its length seems also to vary somewhat, which is partly due to the fact that a constriction may be formed at several different places within it, in which case this weak part in the chromosome will entail a bend there, so that the small arm (apparently) varies in length. It has further been noted that the small arm is relatively largest at early metaphases, whereas at late metaphases it is short and at anaphases sometimes so short that the chromosome behaves as if it were rod-shaped. I am enclined to regard this phenomenon as another indication that the arm is heterochromatic and therefore *later* in its spiralization than the other parts (cf. the above discussion regarding the spiralization of the heterochromatin, p. 36). A consequence of retarded spiralization would in fact be that the arm at early metaphase would seem relatively long, at anaphase, on the other hand, extremely short, when its spiralization has become optimal.

All the other chromosomes of the brown trout are rod-shaped, thus 31 pairs. Their length greatly varies and, as in other species, form an evenly falling size curve, where the largest are about three times as long as the smallest.

Constrictions, which are so common in the chromosome set of the salmon, are scarcely found at all in the brown trout — *nota bene* at metaphase. At early prophase, on the other hand, there are, as usual, numerous constrictions, but it is difficult or impossible to determine their number. They then disappear in the usual way and at metaphase there is often none of them left, sometimes, however, one in the above-mentioned J-shaped chromosome. Moreover, a clear constriction at metaphase can occasionally be observed on one or both of the very shortest rod-shaped chromosome pair, which thus has the same capacity as in the salmon for developing constrictions. Possibly, therefore, these chromosomes in the salmon and brown trout are homologous, seeing that they correspond with one another both in length and in the position of the constriction.

Meiosis. My material consists of two testicles from the Fishery Experimental Station at K ä l a r n e, and the meiosis is passed during the late summer (August).

In the salmon the meiosis was difficult to study and this difficulty, of course, is still more accentuated in the brown trout with its considerably higher number of chromosomes. Fixation in acetocarmine otherwise produces relatively good pictures.

Only the first metaphase of the meiosis has been studied. The bivalents are long and slender and the chiasmata, so far as could be determined, ter-

minal. The number of bivalents in several cases could be determined as approximately 40 and in one case with certainty 40 (microphoto. 8).

There is a certain frequency of pycnotic metaphases, which never seem to proceed to anaphase. It is difficult to determine what proportion of the total metaphases are abnormal, but they may be roughly estimated at about 10—20 %. Before the pycnosis has made the nuclei impossible to study, it can be observed that univalents occur, and large, intensely coloured chromosome knots indicate the presence of complicated multivalents.

Evidently the salmon and the brown trout regularly have a certain number of abnormal first metaphases in their meiosis, when univalents and multivalents occur, although more detailed investigations of this phenomenon cannot unfortunately be made for the present. The discussion regarding the interpretation of these irregularities will be found in the Chapter »Meiotic Disturbances», p. 109.

3. The hybrid Salmo salar × Salmo trutta.

The great difference in number between the chromosomes of the salmon and the brown trout does not prevent hybridization. Such hybrids are sometimes obtained unintentionally in fish cultures, and rather frequently they have been deliberately produced and studied (NERESHEIMER 1937). I am not aware of any certainly known hybrids between these species in nature.

Various experiments, however, have clearly shown that hybridization between the two species is only possible in one direction, namely when the salmon is the mother. Brown trout eggs, on the other hand, could not be fertilized with salmon milt, which indicates that the impediment is of a mechanical nature, *e. g.* the size of the micropyle. No investigations into this problem have, however, been made by the author, as they would have been beyond the scope of this work.

Mitosis. As the salmon has haploid 30 chromosomes, whilst the brown trout has 40, the hybrid should have 70 chromosomes in its somatic mitoses. This is in fact the case. I have examined hybrids from Älvkarleby and Falkenberg, both of which populations, in their mitoses, have that number (microphoto. 9).

The actual chromosome morphology of the hybrid likewise corresponds with that expected (text-fig. 17). Thus, there are 14 V-shaped chromosomes, including (in the Älvkarleby material, cf. p. 47) 2 large hook-like chromosomes, both of which are derived from the mother, *viz.* the salmon. Individual chromosomes among the other kinds are likewise in all probability

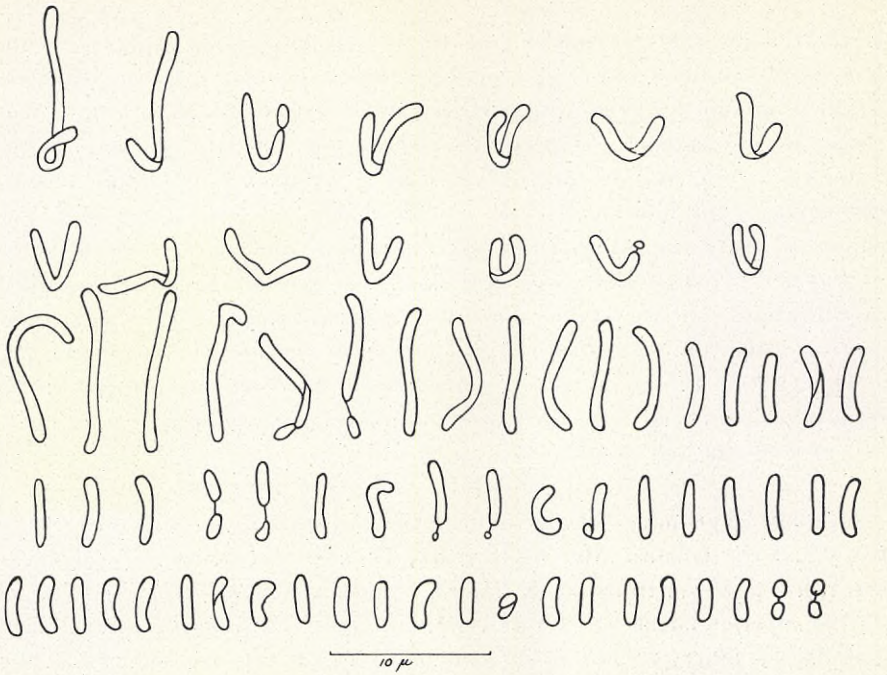


Fig. 17. Salmo salar × Salmo trutta. Mitotic chromosomes.

derived from the mother, namely those which are characterized by constrictions. The salmon, as previously mentioned, has unusually numerous constrictions in its chromosome set, whereas the brown trout has but few. In view of the great variation to which constrictions are always subjected, it cannot be determined whether the hybrid possibly may have more constrictions than the salmon. On the other hand, it is evident that the hybrid has more constrictions in its mitotic chromosomes than the brown trout.

The chromosome size in the hybrid is likewise of interest. It has been previously pointed out that there can be no question of absolute determinations of size in regard to a certain species, so far as concerns the length and breadth of the mitotic chromosomes, seeing that the variations in size are considerable, both intra- and inter-nuclearly. In working with many preparations and examining hundreds of mitoses at different stages, however, one gets a strong impression of the chromosome sizes which are characteristic of the species in question. Though I am fully conscious of the inadequacy of this subjective estimate, it seems fairly certain that the chromosomes of the salmon on an average are the largest among the species examined by me, and that the grayling (Thymallus thymallus, see p. 80) — setting aside the smelt, which is more remote from the other species —, has the

smallest. Thus, the species which has the lowest chromosome number has the largest chromosomes and *vice versa*. That this is a rather frequent occurrence within a group of species is emphasized *e. g.* by DARLINGTON (1937), but numerous exceptions are reported by TISCHLER (1942). According to the same estimate of the size, the hybrid salmon × brown trout seems to have chromosomes of intermediate size relatively to the parent species. The chromosomes of the brown trout have thus been somewhat enlarged in the hybrid. Such changes have been previously observed and have been regarded as indications that the chromosome size, in some measure, is under genetic control. Literature on the subject has previously been cited (see p. 14).

Though the hybrid salmon × brown trout has been previously studied from other points of view, there are, so far as I am aware, no earlier cytological investigations of its mitosis or meiosis.

Meiosis. The males of the hybrid become sexually mature after 4—5 years and the females after 6—8 years. These observations were made at the fish culture institute at K ä l a r n e and were orally reported to me by the superintendent, Dr. GUNNAR ALM, who has also kindly placed such material — otherwise not easily obtainable — at my disposal. According to earlier reports from fish cultures, these hybrids are fertile and their offspring »revert to the parent species» (cf., however, NERESHEIMER 1937, who reports more recent investigations according to which the hybrid is sterile). In view of the peculiar chromosome set of the hybrid, where a very large number of univalents might be expected, I at first regarded these earlier reports with much scepticism. In course of time, however, I found that they were, at any rate, not completely unreliable.

The males pass through the meiosis during the same season as the other autumn-spawning species, namely towards the end of the summer. My material, two hybrid males, was fixed in August.

Owing to the great technical difficulties involved in the nature of this material, it was not possible to study the first stages of the meiosis. My studies were therefore mainly confined to the diakinesis-metaphase stages in the first division.

At these stages a fairly large number of bivalents are formed (microphoto. 10—11). The number of univalents varies greatly from nucleus to nucleus and only in exceptional cases can it be exactly determined: in all cases, however, it seems to range between 10 and 20. In accordance with what is well known from numerous studies of hybrids, univalents occur outside the metaphase plate of the bivalents. A survey of the metaphases is shown in textfig. 18.

Fig. 18. Salmo salar × Salmo trutta. M I showing numerous univalents.

Up to the present I have, unfortunately, not found it possible to determine which chromosomes form pairs and which of them behave as univalents. Nor could it be ascertained whether the latter divide during the first or the second meiotic division. That, as had been expected, they are distributed at random on the daughter nuclei could, however be observed, this being quite distinctly shown by the varying size of the spermids.

The normal bivalents seem, as usual, to have completely terminalized chiasmata. An analysis could be made only on the basis of sporadic metaphases, and then in polar view. Though it is very difficult to determine from this angle whether the chromosomes are univalents or bivalents, successful results were obtained in the following cases.

I	II	III	2 n
14	28	—	70
12	29	—	70
17	25	1	70
10	30	—	70

The last of these metaphases shows only ten univalents, that is, the least possible number if full inter-specific pairing is assumed. This metaphase is fortunately the best and clearest. (Microphoto. 10.)

The occurrence of a trivalent, in conjunction with the existence of trivalents in both species, indicates that intraspecific pairing can also take place. The tendency towards such pairing may be stronger in the hybrid than in the pure species, owing to pairing competition. Even if this source of error is taken into account, the number of bivalents seems to be rather large, thus bearing out the view, based on morphological studies, that the two species are phylogenetically closely related to one another.

Although the spermids have varying chromosome numbers and thus are of markedly different size, all of them seem to be converted into sperms. This seems remarkable, even if it has long been known that the male gametes in animals are not so sensitive to genetic unbalance as in plants (HERTWIG 1936, CONGER 1940).

4. The back-cross hybrid Salmo trutta × (Salmo salar × Salmo trutta).

As considerable practical interest attaches to the forcing of the sterility barrier between the salmon and the brown trout, several attempts have been made at Kälarne to use the F1-hybrid for breeding purposes. Hitherto these experiments have not been successful. So far, merely a few F1-females have become sexually mature in the ponds and they have yielded only a few grains of roe, which, on fertilization with an F1-male, have given a bad result. In fact, merely stray grains of roe were fertilized and the fry soon died.

Somewhat better results were obtained in regard to back-crossing with hybrid males and brown trout females. In such experiments the roe hatched, but the fry died later. Thus it cannot yet be determined whether the sterility barrier is total, or not.

This back-crossing was studied also from a cytological point of view. In these studies two interesting results were obtained, firstly the determination of the chromosome number, in those sperms of the hybrid, that could bring about fertilization, and secondly an insight into the nature of the sterility barrier.

In the preceding chapter it has been mentioned that the meiosis of the hybrid (in the male) shows disturbances, but that nevertheless sperms are developed in large numbers. When brown trout roe is fertilized with this milt, it is found that the fertilization is normal for the salmons, *i. e.* over or round-about 90 per cent. The development seems to proceed quite normally, but the mortality begins to increase beyond normal limits, so that most of the fry die during hatching or soon afterwards. Naturally, also other factors may have come into play. Fortunately, however, the mortality was none up to the stage when I fixed a number of embryos for cytological examination.

In regard to chromosomes, the embryos examined may be divided into two kinds, namely (1) those without (visible) mitotic disturbances and (2) those in which such disturbances are evident.

From the first-mentioned group 14 embryos were selected, where the plates were sufficiently distinct to enable the chromosome number to be determined with almost complete certainty (the varying conformation of the constrictions often causes an uncertainty of ± 1 or 2). These embryos showed the following chromosome numbers:

chromosome number	72	73	74	75	76	77	78	79	80
number of embryos	1	—	4	5	—	3	1	—	—

An additional 10 embryos from the first group, where, however, no entirely reliable plates could be obtained, likewise showed a chromosome number of about 75 (microphoto. 12). Seeing that the brown trout eggs have 40 chromosomes, this shows that the sperms of the hybrid had a varying chromosome number, but that *this variation culminated round the number 35*, with an ascertained variation of 32—38.

No more extensive selection seems to have occurred in the fertilization of the sperms. The embryos which showed mitotic disturbances in several cases also had nuclei with a chromosome number of about 75, whence they had presumably also started with a number in the neighbourhood of 75.

In this way further information can be obtained regarding the meiosis of the F1-hybrid. Seeing that the latter had somatically 70 chromosomes, whereas the bulk of the sperms had 35, it can be inferred that the numerous *univalents as a rule pass through only one division*. Whether this occurs in the first or second meiotic division is, however, as yet unknown. That univalents pass through only one division is normal in plants, but in animals also double division of the univalents occurs. True that rather few data are available in regard to animal hybrids, but several butterfly hybrids, which are the best analyzed, show such a double division. Detailed reviews of the literature will be found in HERTWIG (1936), to which the reader is referred.

The intensive study of the meiosis in hybrids, especially in plants, has clearly shown that univalents in the meiotic division where they do not divide, have great possibilities of not being included in the daughter-nuclei but eliminated. In some cases, owing to their lagging in the anaphase, they tend to disturb that phase, so that a restitution nucleus is formed. In the F1-hybrid salmon × brown trout, however, it seems that most of the univalents, despite lack of capacity for normal passing to the pole, are included with the daughter-nuclei, and that the univalents arrange themselves at random, *i. e.* in the ratio 1:1.

Mitotic disturbances. Many of the back-cross embryos show disturbances in the mitosis. The above-mentioned grouping into undisturbed and mitotically disturbed embryos is certainly not natural, seeing that firstly some of the embryos with disturbed mitoses also showed nuclei with 75 chromosomes, and secondly all the embryos gradually died, this presumably being the result of the previously observed mitotic disturbances. The real explanation seems, instead, to be that at the time of fixation, some of the embryos were more advanced, others less, in regard to the genetic mitotic disturbances, which finally entailed the death of all of them.

The disturbances observed were the following: —

a. Multipolar spindles and nuclei with deviating chromosome numbers.
b. Anaphase sticking.
c. Chromosome elimination.
d. Defective coorientation in time between chromosomes and spindle.
e. Changes in the chromosome morphology.

a. Multipolar spindles and nuclei with deviating chromosome numbers.

Many modern cytological investigations have clearly shown that the spindle, both at mitosis and meiosis, is very sensitive to mechanical, chemical and »spontaneous», *i. e.* usually genetic agents. Multipolar spindles are now so well-known, especially from hybrids, that any literature references on the subject are scarcely needed. I shall content myself therefore by referring to P. HERTWIG (1936) for the zoological and TISCHLER (1942) for botanical material. There is a curious difference between plants and animals in regard to the mitosis, animal hybrids often showing marked mitotic disturbances in the soma, whereas such disturbances are rare in plants. TISCHLER (1942) mentions merely three cases, but BLEIER (TISCHLER, p. 339) points out that such disturbances possibly are more common. As regards the origin of multipolar spindles during mitosis there is also the difference between plants and animals that in the latter they must be attributed to the occurrence of more than 2 centrosomes, whereas centrosomes are missing in the higher plants (TISCHLER 1942) and therefore the organizing of normal and abnormal spindles in plants is as yet entirely unknown. If the difference between plants and animals in the frequency of mitotic disturbances in hybrid embryos proves to be real, at any rate one of the causes of this may be sought in the absence or presence, respectively, of a centrosome.

In the back-cross hybrids there are various cases of multipolar spindles (microphoto. 13—15). The centres for the anaphase movement may be either few or numerous. In all the cases observed, however, the nucleus which showed multipolar anaphase had *considerably more chromosomes than the normal number*. The exact number could not be determined in any case, but it may be roughly estimated at about the tetraploid number, *i. e.* 150.

This observation seems to be of some importance in explaining the existence of multipolar spindles in animals. Two alternative explanations suggest themselves. Firstly, it is conceivable that the division of the centrosomes does not take place synchronously with the division rhythm of the chromosomes, so that *e. g.* a 4-polar, 8-polar, etc. spindle can be formed. In this case there may be a further complication in that the division also of the daughter-centro-

somes is not synchronized, in which case odd-polar anaphases may occur. This explanation implies that the disturbance may effect the *centrosome direct.*

The second alternative explanation is that recently proposed by BÖÖK (1945). He points out that hitherto no tetraploid tissue could be produced in animals with the aid of colchicine, but that multipolar spindles, pycnotic cells, etc. could. If a mitosis for some reason is interrupted at or before metaphase so that no passing to the pole can occur, the result in many cases must be a tetraploid restitution nucleus. *If* the centrosomes had then divided normally, this restitution nucleus will have two centrosomes, which, when the disturbance has ceased and a new prophase commences, will normally divide again. The polyploid nucleus will thus have four centrosomes, normal anaphase movements will be prevented, and a multipolar spindle will result.

Either of these explanations of the origin of multipolar spindles may be applicable to the back-cross hybrids. But, as all such spindles observed also had a high chromosome number, BÖÖK'S explanation seems to be the most plausible.

The consequences of multipolar spindles are obvious. Nuclei are formed with shifting chromosome numbers, produced at random. These nuclei — which is very characteristic of the Salmonoids —, are unexpectedly vigorous and can pass through many mitoses with a greatly reduced chromosome number. In connection with cold-experiments further details on the subject will be given: in this connection the reader is referred only to microphoto. 16, which shows the prometaphase of a nucleus with 37 chromosomes.

b. Anaphase sticking.

TISCHLER (1942) gives a good survey of known mitotic disturbances, from which it appears that anaphase bridges are an often observed disturbance of this nature. Both chemical and genetic agents have been found to be able to produce »stickiness». Nucleic acid starvation also gives such characteristic bridges, as DARLINGTON and LA COUR (1940), GEITLER (1940 d), CALLAN (1942), WICKBOM (1945) and other investigators have found. In their first report, DARLINGTON and LA COUR considered that this stickiness was due to the fact that the chromatids had not divided in the parts in question, but afterwards DARLINGTON and UPCOTT (1941 b) maintained that the phenomenon was due to the fact that cold had induced the heterochromatin to »sister fusion». In this latter work they give a valuable literary review of reports of such mitotic bridges that have no manifest cause and are therefore called spontaneous. According to their later interpretation, this stickiness is due to chromosome breaks followed by reunion between sister chromatids.

In the back-cross hybrids there are numerous cases of anaphase sticking (microphoto. 17—21). This incapacity for normal anaphase separation may affect one or more of the chromosomes and be more or less accentuated. In certain cases clear bridges — which, though the centromeres cannot be observed, give a decided impression of being bicentric chromatids —, are produced. Occasionally also short chromatids are left in the plate in such a position that they may be suspected of being acentric.

In other cases, on the other hand, where there are undoubtedly two centromeres, whose chromatids are separated only in part, it cannot be determined with certainty whether we are concerned with an abnormally *retarded* anaphase separation, or whether the chromatids in some place have undergone secondary (lateral) fusion, so that the anaphase movement is mechanically prevented. Possibly a »pseudochiasma», *i. e.* a nonsister chromatid reunion (DARLINGTON and UPCOTT 1941 b) may furnish the explanation of such a picture. No acentric chromatid, however, can be observed in such a chromosome configuration.

It is quite conceivable that such an impeded anaphase separation sometimes leads to the formation of restitution nuclei, which afterwards might give rise to multipolar spindles in the next nuclear generation. Polyploid prophases have also been observed, but it cannot be determined how they have been produced.

c. Chromosome elimination.

In the description of non-disjunction (p. 43) it has already been mentioned that the borderline between non-disjunction and chromosome elimination is fluid. The primary reason why some chromosome meets with one of these two events is undoubtedly that its centromere is either unable to arrange itself together with the others in a normal metaphase plate, or else that it arrives there so late that normal anaphase movement of the chromatids is prevented. Naturally also the capacity of the centromere for division simultaneously with the other centromeres may be reduced or absent.

It is of interest to note that one or more centromeres in a nucleus may be abnormal without the others deviating from the normal behaviour, which implies that the centromeres have a certain measure of individuality (cf. p. 99). Microphoto. 22 shows two such chromosomes, the centromeres of which show a different behaviour.

Chromosomes whose centromeres do not behave normally, however, seem for the most part to be included in one of the daughter-nuclei, whence non-disjunction seems to be commoner than chromosome elimination.

d. Defective coorientation in time between chromosomes and spindle.

The orientation of the chromosomes may also be disturbed in time, not merely in space. We find some metaphases where the orientation of the chromosomes, a distinct spindle and so on, clearly mark the metaphase, whereas the state of contraction of the chromosomes is not metaphasic. Sometimes they are long and narrow as in the prophase and in that case clustering together in a maze, sometimes so strongly contracted that they are almost spherical and look like fragments. Seeing that such cells, as also all the other mitotic disturbances, lie scattered among normal cells or those with other disturbances, there can be no question of external action on the chromosomes (microphoto. 14).

e. Changes in chromosome morphology.

In addition to the morphological deviations which affect all the chromosomes of the whole nucleus and which presumably may be attributed to spiralization in different degree, also other morphological changes, which can scarcely be explained in this way, are observed. In microphoto. 23 we see for example a chromosome of immense length, with »terminal» centromere, which is just about to divide, as is clearly visible in the microscope. No such chromosome is found in the normal chromosome set of either the salmon or the brown trout. This chromosome had in all probability been produced by the breakage of two chromosomes and following reunion, so that a monocentric giant chromosome and a small acentric chromosome had been formed. The latter had afterwards probably disappeared. In microphoto. 23 we see that the chromosome in question, which is almost as long as the entire spindle, had not been able to orientate itself normally in the plate. That chromosome breaks can occur numerously is shown by microphoto. 15, where a metaphase consists almost solely of fragmentary chromosomes.

Summing up, it can be noted that these back-cross hybrids show such marked mitotic disturbances that their early death could be foreseen. The primary disturbances which had occurred were *chromosome and chromatid breaks* (anaphase sticking, new chromosomes, possibly multipolar spindles as a consequence of anaphase sticking), *inefficient centromeres* (some sort of anaphase sticking or lagging chromosomes, elimination) and *inefficient centrosomes* (no coorientation between spindle and chromosome cycle, possibly multivalent spindles).

Three fundamental conditions for the successful course of a mitosis were thus missing in many of the embryonal nuclei. What is the explanation of

this? No external influences can have been involved, seeing that the material had been treated exactly in the ordinary routine way and even concurrently with other, normal embryo material. It must also be noted that the *F1-hybrid salmon × brown trout does not show any such mitotic disturbances.* Cytoplasmic factors in this case are out of the question: the disturbances must have been caused by the unbalanced chromosome set entailed by the sperm.

It is thus evident that the mitosis is under the control of special gene systems, which in this hybrid did not operate harmoniously and normally. This question is discussed in Chapter IV E (p. 94).

5. The Char (Salmo alpinus L.).

Also this Salmonoid is a common fish in Sweden, especially in the northern parts. In some large lakes in the south of Sweden such as Vättern, Sommen, etc. the char occurs as a survival from the epoch after the melting of the ice, when the waters in the southern parts of the country were considerably colder than at present. In the larger lakes the char occurs in a large light-coloured form, which is called »storröding» (big char) or *Salmo alpinus salvelinus.* In several places in northern Sweden we find the »fjällröding» (mountain char), which is smaller and darker and is sometimes called *Salmo alpinus alpinus.* There are, however, some other forms, including dwarf forms in certain lakes in the mountainous districts, which, however, have not yet been named. As regards these names the remarks previously made about the taxonomy of the brown trout are applicable. Material was obtained of three different types, namely the big char from the lake Vättern, the mountain char from the lake Stor-Uman in Lapland and the dwarf char from the lake Järpen in Jämtland.

The somatic chromosomes of the char have already been briefly described in connection with the discussion regarding changes in chromosome size, constrictions etc. (text-figs. 1—4). The diploid chromosome number, as in the brown trout, is 80 (microphoto. 24).

Also the chromosome morphology is very like that of the brown trout. All the eight pairs of V-shaped chromosomes are fairly similar in size and equal-armed. The hooks which are characteristic of the salmon are thus missing. The rod-shaped chromosome of the brown trout with a short heterochromatic arm is likewise missing in the char. The 32 pairs of rod-shaped chromosomes show, as usual, an evenly falling size curve. In contradistinction from the brown trout, however, constrictions occur, to some extent persisting till late metaphase, in which the char resembles the salmon. One of the larger

pairs of rod-shaped chromosomes has usually a well-developed constriction, which is situated proximally; this pair, however, is not always morphologically distinct, as is a corresponding pair in *Salmo fontinalis,* the American relative of the char.

The three populations of the char examined by me, so far as I am aware, the only ones that have hitherto been cytologically studied —, have been isolated from one another for a considerable space of time. According to MAGNUSSON and GRANLUND (1936), Lake Vätter was isolated from the sea during the last period of the so-called Yoldia Sea, that is, about ten thousand years ago. About two thousand years later the surface of Lake Vätter again was in contact for a short time with the sea, now with the so-called Ancylus Sea. Whether the present stock of char in the lake Vättern had been isolated for ten or eight thousand years is therefore not quite certain, but a period of eight thousand years is evidently a minimum. It is therefore of interest to note that no definitely observable differences in chromosome morphology are found between the populations examined by me. True that the population from the lake S t o r-U m a n shows an indistinct constriction on the largest rod-shaped chromosome pair, whereas in the char of Lake Vätter this chromosome pair never shows constrictions. But, in view of the above-mentioned variation in the configuration of the constrictions, no great importance can be attached to this difference. As was the case with the salmon isolated in the lake Vänern, this is an *example of a chromosome set which had not undergone any morphological changes, visible in the microscope, during an isolation of eight thousand years.*

Testicles were fixed at K ä l a r n e in J ä m t l a n d in August. As regards the meiosis of the char, it differs in no essential respect from that of the brown trout. I have unfortunately not been able with complete certainty to analyze any M I, but several metaphases have shown about 40 bivalents, whence this number seems to be the normal one. I can, however, state with complete certainty that meiotic disturbances occur with some frequency also in this species, manifesting themselves in observed univalents, and in one case also a trivalent in the form of a chain. The chiasmata, in the cases where they could be studied, were terminal.

6. The hybrid Salmo trutta × Salmo alpinus.

This hybrid is at present being studied at the Kälarne Fishery Experimental Station, especially with regard to its capacity for competing with the parent species. In exceptional cases malformations, probably due to

mitotic disturbances, have been found to occur in the fry. Also the differentiation of the gonads is somewhat disturbed, which, however, does not seem to be due to mitotic disturbances.

Meiosis. The material was fixed in August. Seeing that the salmon and the brown trout, despite their entirely different chromosome number, show a relatively marked interspecific pairing in the hybrid between them, we might be tempted to suppose that the hybrid brown trout × char would show a more complete pairing, seeing that not only the chromosome number, but also the chromosome morphology is so similar. This, however, is by no means the case; the lack of pairing, on the contrary, is very pronounced.

The first metaphase of meiosis shows such marked disturbances that it passes into a modified anaphase only in exceptional cases. In the great majority of cases the metaphase remains stationary, the spindle is increasingly elongated and at last the chromosomes become pycnotic and the cell dies. The formation of sperms is completely absent, whence the male is quite sterile. The female meiosis has not been studied, and no hybrid female has hitherto had mature eggs.

The usual technical difficulties in the study of the meiosis are quite as great in this hybrid, as the chromosomes tend to become pycnotic and as the univalents are so numerous that they arrange themselves in accessory metaphase plates on both sides of the normal plate. This prevents all study in polar view. It is thus very difficult to reckon the number of univalents and the visible number is very seldom less than ten; however, to judge by the analyses of the hybrid salmon × brown trout, quite as many univalents probably lie concealed in the compact metaphase plate. The whole number of univalents seems to be about 20—30, which gives 25—30 bivalents. Possibly, however, also multivalents occur.

The microphoto. 25 shows a typical metaphase with the univalents accumulated outside the normal metaphase plate. That the spindle is elongated when unpaired chromosomes occur is wellknown; DARLINGTON (1937) and TISCHLER (1943) have given many examples of this.

Although the meiosis of the female has not yet been studied, we may venture to infer that the chromosomes of the brown trout and char, despite the marked resemblance in the external morphology, are in fact greatly differentiated from one another. The chromosomes of the brown trout, therefore, in spite of differences in external morphology and a striking difference in number, seem to be more homologous with those of the salmon than with those of the char. This also clearly corresponds with the generally adopted systematic position of these fish.

7. The speckled trout (Salmo fontinalis Mitch.).

The speckled trout or brook trout as it is also called, does not occur in the wild state in Sweden, but has been naturalized in some lakes and is otherwise found mostly in ponds.

Mitosis. The speckled trout has previously been subjected to cytological investigation, in so far as PROKOFIEVA (1934) has studied the hybrids between this species and *Salmo salar* and *Coregonus baeri*. In the somatic mitoses she found 70 and 80 chromosomes, respectively, from which it may be inferred that *Salmo fontinalis* has a chromosome number of 2 n = 80. Owing to the nature of the material, PROKOFIEVA was unable to make a close study of the chromosome morphology in the speckled trout, but she found that the species must have some chromosomes with satellites, since such chromosomes occurred in the hybrid with *Coregonus*, which has no satellite-chromosomes.

My speckled trout material comprises, as usual, embryos aged about 3 days. Unusually distinct metaphase pictures of this species were obtained. It was found that PROKOFIEVA'S number was not correct. The speckled trout has in fact 84 chromosomes in its embryonal cells (microphoto. 26).

As PROKOFIEVA gives the number 84 for the brown trout, which in fact has 80 chromosomes and the number 80 for the char, which actually has 84 chromosomes, some confusion of the material seems to have occurred. This is by no means inconceivable, seeing that the colour of the roe may vary considerably, so that it does not clearly indicate the species. However, her statement regarding the occurrence of satellites in the speckled trout argue against this explanation. As previously mentioned, the brown trout has remarkably few secondary constrictions, whereas the speckled trout, relatively speaking, has many of them and especially a chromosome pair in which a secondary constriction of unusual length occurs. The morphology therefore indicates that she had counted wrong, owing to bad pictures of both species.

The chromosomes of the speckled trout are of the usual *Salmo* type (text-fig. 19), though unusually well differentiated. V-shaped chromosomes, as in the brown trout and char, occur in 8 pairs. Two pairs of these V-shaped chromosomes have arms of markedly different length. At any rate one of these unequal-armed pairs has frequently a secondary constriction in the short arm. The V-shaped chromosomes have a remarkably uniform size.

The rod-shaped chromosomes number 34 pairs. Also in regard to them the differences in size are equalized, so that they do not show such striking

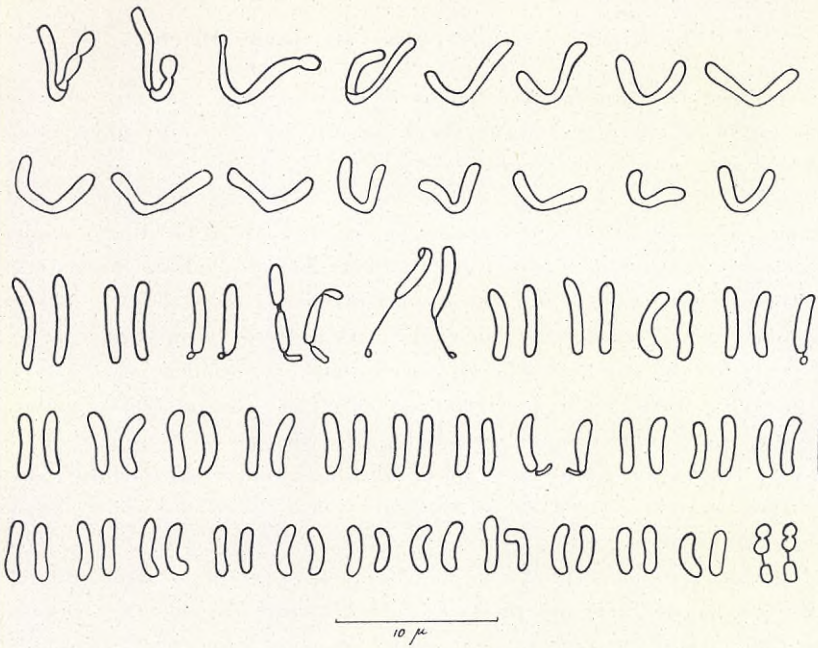


Fig. 19. Salmo fontinalis. Mitotic chromosomes.

differences between large and small as in the species previously described. The longest rod-shaped pair has a constriction, which is usually rather distinct, near the centromere.

Another rod-shaped pair, of intermediate size, has a second very short arm and is thus two-armed. This short arm, however, does not attain the normal chromosome breadth and is also distinctly lighter in colour. I consider it probable that this little arm is heterochromatic, as was the case with one chromosome pair in the brown trout. Yet another of the larger pairs has an extremely elongated constriction, which is situated very proximally. This chromosome pair is extremely characteristic of the speckled trout and, in contradistinction from other constrictions, they seem to be always well-developed, though they show a considerable variation in length. Also the proximal part of the chromosome, where the centromere lies, is variable. Sometimes it is distinctly set-off as an elongated small chromosome arm, sometimes it is short and contracted like a ball. If PROKOFIEVA had seen this chromosome, it seems to be here that she had noted her »satellite».

Yet another of the larger rod-shaped pairs has a distinctive appearance. The centromere probably is situated quite »terminally», but this is difficult to determine, as there is a distinct constriction proximally. Then follow in a

distal direction a chromosome part of normal colour and size, then again a constriction and a distal part. The proximal and distal parts both have a lighter colour and a smaller diameter. Probably we are concerned also here with heterochromatic parts. Owing to the varying configuration of the constrictions, this chromosome pair varies considerably, so that I long suspected it to be a pair of sex-chromosomes; but this does not seem to be correct. In any case no decisive cytological evidence of this can be adduced.

Finally, it may also be mentioned that the smallest rod-shaped chromosome pair has a median constriction and often indications of another in one arm. This chromosome pair thus shows unmistakable resemblances to a corresponding pair in the salmon and brown trout.

Hitherto I have not studied the meiosis. Suitable stages were not found, but merely spermatogonial metaphases, where the chromosome number could be ascertained only with difficulty. These chromosomes, as usual in such cases, are of an entirely deviating form, being extremely short and contracted, so that they almost assume a spherical appearance.

8. The hybrid Salmo fontinalis × Salmo trutta.

This hybrid also has been subjected to growth studies at the Kälarne Fishery Experimental Station. The hybrid shows great resemblances to the hybrid *Salmo trutta* × *Salmo alpinus,* both as regards malformations in the fry and also the meiosis pairing. My material was fixed in August.

Meiosis. The number of univalents is so large that no analyses could be made of the frequency of bivalents, univalents and possible multivalents. As in the hybrid *Salmo trutta* × *Salmo alpinus,* the univalents arrange themselves around the metaphase plate, where they form accessory plates. Possibly they are hindered from proceeding to metaphase by the fact that the spindle at an early stage is constricted and becomes immensely long, whence it is often bent. The number of univalents appears to be actually still larger than in the previously described hybrid (microphoto. 27). No figures, however, can be given, as it is impossible to count the univalents, seeing that the metaphases, probably owing to defective pairing, tend very soon to become pycnotic. The first metaphase of meiosis degenerates and no sperms are formed. There cannot be any dobt that, in the male, a complete sterility barrier separates the speckled trout from the brown trout. In the females the conditions are not so well known, but certain preparatory investigations, seem to show that also in the females, the development of the eggs is stopped at a very early stage. I am not aware of any hybrids under natural conditions.

9. The Gwyniad (Coregonus lavaretus L.).

The species and subspecies of the gwyniad have long been the real crux of the taxonomists. Also the most modern taxonomist (Wagler 1941) reckons with several species within the European area. The gwyniad problem has many interesting aspects from an evolutionary point of view, and I hope elsewhere to be able to give a more extensive report on the existing facts. Here I shall confine myself to referring to what I have already pointed out, in the discussion on the so-called subspecies of the brown trout, regarding the classification of the fishes.

Seeing that the variation of the gwyniad in different lakes and even in the same lake is still more marked than in any of the other Salmonoids, and as the same seems to be the case also with the Coregonids of the New World (HILE 1935), it is of very special interest to study the appearance and number of the chromosomes in the species. It was, of course, conceivable that in this way the taxonomy of the different forms might be elucidated. It also seemed not improbable that some cytological explanation could be obtained in regard to the ultimate causes of the observed variation.

As a step in this investigation, I have therefore examined the roe material of several different populations of the gwyniad. My material is the following:

1. Gwyniad from Lake Vätter of *oxyrhynchus* type. The snout greatly elongated.

2. Gwyniad from the Baltic off Älvkarleby. *Lavaretus* type, very large.

3. Gwyniad from the lake Gardiken. Vilhelmina district, Lapland, at the border of the area of distribution of the gwyniad. This form, however, seems to be naturalized, though the origin is unknown.

4. Gwyniad of the ordinary lake type, »big gwyniad» from the lake Ansjön, Jämtland.

5. Gwyniad from the lake Wian, Blekinge. Relatively small (only in cold experiments).

Among the Salmonoids investigated, the gwyniad showed the greatest deviations from the normal. That non-disjunction is an almost normal occurrence in this species has already been reported (p. 42). In addition, there are embryos with a greatly deviating chromosome number and a new type of chromosomes, namely fragments. A description of the normal mitotic chromosomes of the gwyniad will be found below under the head of mitosis, followed by sections on unbalanced embryos and embryos with fragments.

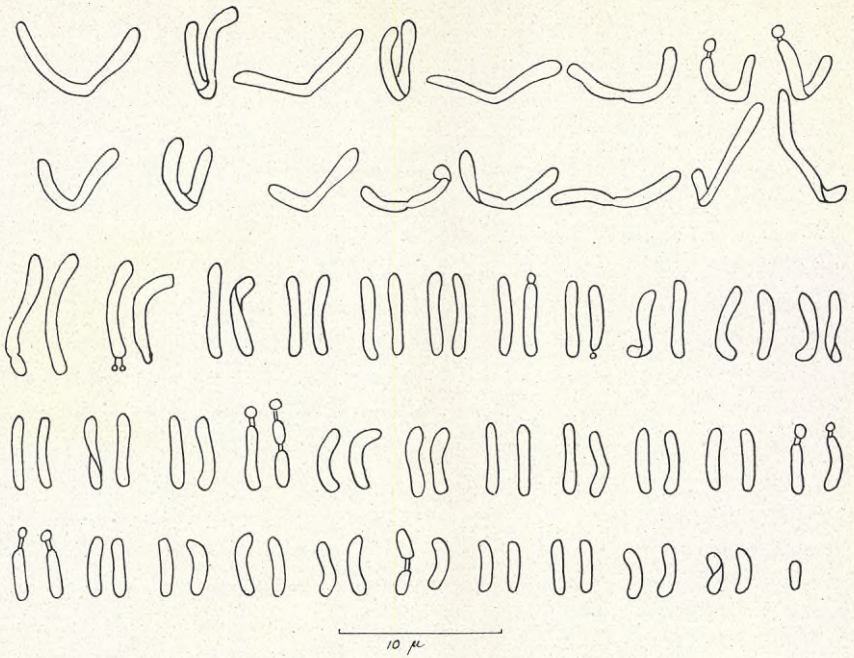


Fig. 20. Coregonus lavaretus. Mitotic chromosomes.

Mitosis. PROKOFIEVA (1934) studied *Coregonus lavaretus baeri*, KESSLER and found that this species had 80 chromosomes in its embryonal cells. Her material was fixed at an age of five days, mine, on the other hand, at an age of two days. Despite the fact that the chromosomes, according to PROKOFIEVA, as a rule lay rather scattered, so that they were easy to count, she had to admit that »chromosome differentiation is achieved in this species with difficulty» (p. 501). She proposes the following preliminary grouping of the chromosomes in morphological groups: long, equilateral, *i. e.* V-shaped, six pairs. Long, inequilateral, *i. e.* hook-like or J-shaped, two pairs. Next come the rod-shaped, *i. e.* »headed», chromosomes of varying length.

I can confirm the statement that the diploid number of chromosomes in *Coregonus lavaretus* is 80 (microphoto. 28). This number in fact is that usually found. This, however, does not exclude the occurrence of variation, which must be all the more marked as several nuclei with the same number have not the same chromosome morphology. As previously mentioned, this is due to non-disjunction. In this way various difficulties are encountered in determining the normal morphology of the 80 chromosomes (text-fig. 20).

The normal number of V-shaped chromosomes, as PROKOFIEVA found, seems to be 16, that is, 8 pairs. This number is also the same as in the species

previously described, with the exception of the salmon. One of these V-shaped pairs, moreover, often has a constriction distally in its longest arm. Besides this, among the V-shaped chromosomes, we find some that are more J-shaped, that is, where the one arm is distinctly longer than the other. Also in this respect I am enclined to agree with PROKOFIEVA'S statement that there are two pairs of such chromosomes. Sometimes, however, they are completely missing, although the entire chromosome number is 80.

Among the rod-shaped chromosomes of varying size according to the usual Salmonoid scheme, there are several which have constrictions. As a rule the latter are very distinctly situated at the distal end of the chromosome, which cannot be said to be so regularly the case with any other Swedish Salmonoid. It is chiefly chromosomes of intermediate size among the rod-shaped that carry such constrictions. In the prophase they are, as usual, much more common.

The five studied types cannot be stated with certainty to differ in chromosome morphology, but several of them show minor dissimilarities. To determine them, with the perpetually occurring non-disjunction, is, however, unfortunately almost impossible.

Unbalanced embryos. At the very outset of my studies of the Salmonoid chromosomes I found a gwyniad embryo, which proved to be a mosaic of cells containing 20, 40 and 80 chromosomes. The 20-chromosome nuclei were by far the most numerous. They divided rapidly and showed many metaphases (SVÄRDSON 1941). These nuclei with a chromosome number below the haploid number were then considered to have arisen by some kind of somatic reduction, especially as the number was half of the haploid.

Subsequently this find was shown not to be unique, but characteristic of a type of abnormal embryos, which occur here and there among examined gwyniads. The first consignment of roe containing this embryo was packed in a wooden box in which the roe lay on frames. At the bottom of the box a piece of ice had been placed, to keep the temperature down. This is in fact the usual way of transporting gwyniad roe for practical fish culture. It was therefore plausible to suppose that the abnormal embryo had been produced by a cold shock. Other transports, without ice, were therefore arranged for purpose of control, besides which direct cold experiments with gwyniad were also conducted.

Although the phenomenon is essentially the same as that observed after cold shock, it was found that a number of mitotic disturbances also occur spontaneously in many gwyniad embryos. Embryos showing mitotic disturbances occur amongst series of others fertilized at the same time and, in

every respect, treated and fixed in the same way. The cause of the disturbances is thus evidently of genetic nature.

The nature of the disturbances is, in many respects, like that previously reported in regard to the back-cross hybrids *Salmo trutta* × *(Salmo salar* × × *Salmo trutta)*. Anaphase sticking is thus common and sometimes results in abnormally lengthy chromatids at anaphase, which in this position are subject to telephase changes, vacuolization, etc. (microphoto. 29). In this way binucleate cells are occasionally produced, but it is uncertain whether these nuclei, during a later division, coalesce into a polyploid nucleus, as was found *e. g.* by DOZHANSKY (1933). Polyploid nuclei have been observed merely in isolated cases. This is possibly due to the rare occurrence of multipolar spindles. In this respect there seems in any case to be a difference in degree as compared with the previously mentioned disturbances in the back-cross hybrids.

In one case it has been observed that anaphase sticking has entailed a cleavage of the centromere at anaphase (microphoto. 30—31). This seems to bear out the view that the chromatid at anaphase is double. This still controversial question has recently been discussed by WICKBOM (1945), to whose work the reader is referred.

The most common form of disturbance is that a few chromosomes are left in the metaphase plate. In many cases non-disjunction then occurs (microphoto. 32), but probably still oftener chromosome elimination. In this way the number of chromosomes is reduced and the nucleus in the next mitosis is subjected to further disturbances, such as deficient co-orientation in time, varying spiralization in the spindle, tendencies to clumping, etc. Whether such cells can recover their balance and again begin to divide at more or less normal mitoses, is uncertain. It is a fact, however, that cells with different chromosome numbers can be observed in typical mitoses, where, so far as can be judged, no disturbances occur. It would be extremely interesting and important for an analysis of these conditions if it could be ascertained whether these cells had been produced direct by multipolar anaphases, or whether they had developed »step by step» through repeated chromosome elimination. In many cases such nuclei have a chromosome number of about 20. It seems more probable that they have been produced all at once owing to multipolar spindles. Such nuclei can certainly pass through several mitoses in succession, seeing that several metaphases with the same chromosome numbers can be observed in close proximity to one another. In cold treatment this is a still commoner observation.

As the chromosomes tend to clump and in general to behave in such a way that their number cannot be determined with certainty, no direct estimates

can be made regarding the frequency of the occurrence of nuclei with varying numbers. Among the nuclei in regard to which the chromosome number could be determined with some degree of certainty, however, nuclei with numbers about 20 and 40, respectively, preponderate. The latter, of course, may simply be derived from haploid nuclei, but the former, that is, those with a number roundabout 20, must have arisen after the fertilization.

This tendency, which is observed also in those nuclei with a varying chromosome number that are produced in cold treatment, is of considerable importance for understanding the chromosome phylogeny of the salmons.

The frequency of *embryos* in which disturbances occur in some form is likewise very difficult to determine, seeing that a large number of nuclei may be normal, with the usual number of chromosomes, whilst a single nucleus may deviate in some part of the embryo. This, indeed, is not a very common event, as mitotic disturbances as a rule occur in several nuclei, but it conduces to make an estimate of frequency a very laborious and difficult task. My figures, which must be regarded as very approximate, work out at 10—20 per cent. In certain populations disturbed embryos seem to be more common, in others more rare. They occur, however, in all the forms studied and must thus be regarded as typical of the gwyniad.

Embryos with fragments. The determination of the chromosome number of the gwyniad was not so easy as the good metaphase pictures might lead us to suppose. This was due partly to non-disjunction, partly to the occurrence of a very small chromosome — a fragment (microphoto. 33).

It was found that this fragment occurred very irregularly. Within the same embryo it might be single, twofold or even threefold, whilst it was completely missing in some nuclei. It varied also in form, so that ossasionally, owing to developed constrictions, it was nearly as large as the smallest of the normal chromosomes.

The fragment was evidently centric and, in course of time, its irregular distribution was revealed, with complete certainty, to be due to non-disjunction. It was also ascertained, that the fragment occurred in *every form of gwyniad investigated.* Moreover, it usually occurred singly, and only in about half of the embryos examined. An estimate of frequency showed that 40 per cent. of the embryos were devoid of fragments. *The fragment thus occurred in about half of the embryos and as a rule singly.*

Heterochromatic fragment chromosomes such as this have been found in a number of organisms, especially in plants. DARLINGTON (1937) gives a table (p. 145) in which 9 animals and 29 plants with supernumeraries are included. Additional data are given by DARLINGTON in a later paper (1939)

as well as by DARLINGTON and UPCOTT (1941 a) and MÜNTZING (1944). According to DARLINGTON and UPCOTT, the fragments may have a physiological function which, by natural selection, tends to preserve them, thus counterbalancing the tendency to loss which they show at mitosis and meiosis.

These supernumeraries of ordinary type are heterochromatic and variable in form as in the gwyniad, but do not regularly occur in such frequency as in that fish. Particularly instructive is the distribution of supernumerary fragment chromosomes in rye. MÜNTZING (1945) found that the fragments showed marked non-disjunction at meiosis, so that crossing between individuals without fragments and those with one fragment produced but few progeny with one fragment. As regards the gwyniad, the occurrence or absence of the fragment must be due to segregation in one of the parents which is a heterozygote in regard to the fragment. The fragment thus seems *not to be an ordinary supernumerary but, instead, a sex-chromosome.*

Sex-chromosomes in fishes have been the subject of much discussion both genetically and cytologically. A number of authors report the observance of cytologically recognizable sex-chromosomes in fishes, namely

GEISER (1924)	in	*Gambusia holbrooki*
FOLEY (1926)		*Umbra limi*
VAUPEL (1929)		*Lebistes reticulatus*
RALSTON (1934)		*Platypoecilus maculatus, P. couchiana* and *Xiphophorus helleri*
BENNINGTON (1936)		*Betta splendens*
BARRIGOZZI (1937)		*Cyprinus carpio*

As for *Lebistes reticulatus,* on the other hand, WINGE (1922), IRIKI (1932) and WICKBOM (1943) have found no indication of the occurrence of morphologically recognizable sex-chromosomes nor of chromosomes which are shown by their behaviour at meiosis to be sex-chromosomes.

Platypoecilus and *Xiphophorus* have been examined by FRIEDMAN and GORDON (1934) as well as by WICKBOM (1941, 1943), with the same negative result.

SVÄRDSON and WICKBOM (1942) have examined *Betta splendens* without finding any sex-chromosomes.

Cyprinus carpio has been controlled by MAKINO (1939 a) with negative result.

These contradictory results have been thoroughly discussed by WICKBOM (1941, 1943). He comes to the conclusion that the irregularities at meiosis

which had been regarded by certain earlier authors as indicating the occurrence of heterochromosomes can very well be explained by random distribution of chiasmata, the different size of the chromosomes, etc. WICKBOM (1943, p. 21) accordingly is forced to the conclusion: »Hitherto there is no cytological evidence for the presence of heterochromosomes in Teleosts». Nevertheless, it must be pointed out that the presence of heterochromosomes is unquestionably indicated by the frequent occurrence of a perfectly normal sex-ratio. Certain genetic studies on the sex-chromosomes of the fishes (see p. 114—117) seem, however, to indicate that they deviate from hitherto known rules, and they may possibly be regarded as not yet markedly differentiated morphologically. WICKBOM'S view is shared also by MAKINO (1939 a) and GALGANO (1941).

Anura and *Urodela* likewise fail to show any distinct, cytologically recognizable, sex-chromosomes (WICKBOM 1945). The numerous cases in which the occurrence of such sex-chromosomes is reported in the literature may be attributed, according to WICKBOM, to misinterpretation of the position of the chiasmata, and other such errors. In fact, there seem to be no reliable data regarding the existence of cytologically recognizable sex-chromosomes among the lower Vertebrata.

It is indeed conceivable that a chromosome fragment in a gwyniad embryo, owing to non-disjunction in one of the first divisions, may have vanished from the major part of the embryo. It is incredible, however, that out of the large number of embryos (about 70) of different forms which I have thoroughly examined, nearly half should have lost their fragment, so that I was unable to find it in any mitosis. The number of mitoses examined in each embryo varied between 15 and 20, depending on the preparation. This forces me to the conclusion that the fragment must be regarded as a more or less heterochromatic sex-chromosome. *The existence of cytologically recognizable sex-chromosomes in the fishes and the lower vertebrates in general has thus for the first time been demonstrated.*

Seeing that the migration of the gwyniad into Sweden may be estimated to have occurred about ten thousand years ago and as most of the populations have been more or less separated since then, it must be designated as remarkable that this little heterochromatic fragment should have retained its size in all the populations. The probability that it had developed independently in each population seems to be infinitesimal. This is thus yet another indication that the chromosomes have a marked morphological constancy in the species examined.

If the fragment is actually a Y-chromosome, non-disjunction should comparatively often entail hermaphroditism or disturbances of some kind, since

the regular occurrence of the fragment shows that it contains more or less important genes. Hermaphrodites with testicles and ovarial tissue in the same fish are in fact known as regards the gwyniad, but scarcely on any large scale. Whether they occur more frequently in the gwyniad than in other salmons is not known.

There are no reliable bases for judging which chromosome is the homologous chromosome of the fragment. This can only be determined by studying the meiosis in the heterogametic sex. Which of the sexes is the heterogametic in these fishes is not yet definitely clear.

As for the meiosis of the male, I have so far studied it merely with an unsatisfactory fixing technique, namely on Bouin-fixed material. No definite signs of the presence of fragments could be detected. This, however, does not mean very much. By cold shock treatment I managed to obtain both haploid and triploid gwyniad embryos. These data (see p. 94) strongly bear out the view that the female is heterogametic. Assuming the correctness of MRSIC'S results (1923), according to which overmature eggs of *Salmo* yielded a surplus of males, not fully mature eggs, on the other hand, a surplus of females, these findings can be explained by the supposition that the Y-chromosome passes into the polar body or not, depending on the temperature and other conditions. In my view, the only possible explanation is that the female in such a case must be heterogametic. This, of course, refers to *Salmo* and does not necessarily apply to *Coregonus*. Although the question is still open, there are various indications that the *female* is the heterogametic sex in *Coregonus lavaretus*. I hope subsequently to be able to furnish definite proof of this, with the aid of crossings with species that certainly have no fragment.

10. The small Gwyniad (Coregonus albula L.).

The taxonomic position of this species is very obscure. WAGLER (1941), who divides *Coregonus lavaretus* into 6 species solely in the central European area, maintains that *Coregonus albula* is a more independent form. In Sweden, however — where all forms of the gwyniad are at present combined in a single species —, *C. albula,* the whitefish or small gwyniad, is regarded as a separate species. The features which mark it out, however, are somewhat vague, now that all distinctive characters from the original diagnosis have been eliminated, except one. All forms of *Coregonus* that have the lower jaw distinctly longer than the upper are now regarded as *C. albula.*

As to whether the classification of the small gwyniad as a distinct species is justifiable or not, I will not for the present express an opinion. Special

experiments with crossings between *C. lavaretus* and *C. albula* have, however, been started, with a view to the elucidation of this question.

My material of *C. albula* comprises embryos from the lake Mälaren as well as from the lake Sommen in the south of Sweden. The small gwyniad has not previously been cytologically studied.

The chromosomes show great resemblances to the other species (text-fig. 21). Thus, the diploid number of chromosomes is 80, as in the gwyniad proper, the brown trout and the char (microphoto. 34).

Also the morphology is the same as that previously reported. Thus, the small gwyniad has 8 pairs of V-shaped chromosomes and 32 pairs of the rod-shaped. Out of the V-shaped chromosomes, two pairs have arms of markedly different size, the longest arm being about twice as long as the short one. Not more than one or two pairs have an exactly median centromere. The V-shaped chromosomes — in the material hitherto examined —, have not shown any constrictions.

The rod-shaped chromosomes have a somewhat smaller number of constrictions than in the studied populations of the gwyniad proper. Thus, at most two chromosomes (not in pairs) in the same plate were provided with constrictions. They are situated distally, as in the gwyniad.

Thus, although my *C. albula* material is rather limited, it seems that the chromosome morphology may possibly enable us to distinguish between the gwyniad proper and the small gwyniad. That the species are closely related to one another is also indicated by the rather common occurrence of non-disjunction. Nuclei with chromosome numbers over (or slightly under) 80 thus occur to a certain extent. My previous report (SVÄRDSON 1941) regarding the existence of 82 chromosomes in the small gwyniad was occasioned by some excellent plates of that nature. In the hybrid between the gwyniad proper and the small gwyniad, nuclei with 84 chromosomes have recently been found. Also in the small gwyniad some cases of nucleic acid starvation, nuclei with unbalanced chromosome numbers as well as multipolar spindles have been observed.

The two forms of *Coregonus* probably differ in regard to the occurrence of fragmentary chromosomes. Thus, in most of the preparations examined I have been unable to find any typical fragment. On the other hand, in a single nucleus I found a typical chromosome fragment, which, however, was somewhat larger than that of the gwyniad proper. As it did not occur in the adjacent nuclei, it had in all probability been produced by chromosome breakage in previous nuclear generations.

Another embryo had fragments of all sizes in many nuclei, but this embryo was quite abnormal. Several of the nuclei seemed indeed to be normal, with

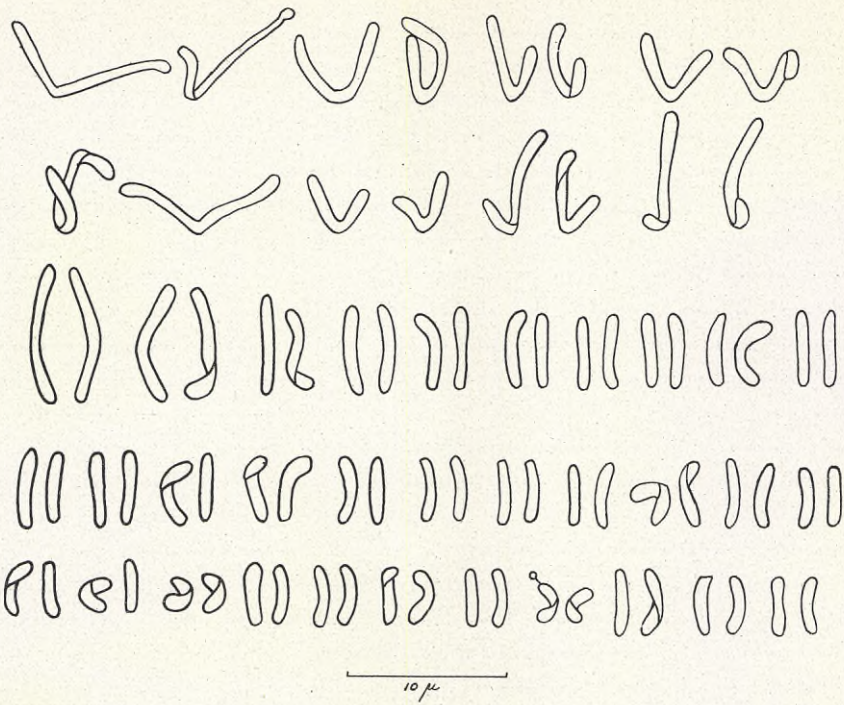


Fig. 21. Coregonus albula. Mitotic chromosomes.

the diploid number of chromosomes, but others had greatly varying chromosome numbers and chromosomes of new morphology. The obvious explanation was that the chromosomes in this embryo had an unusually marked tendency to breakage. Microphoto. 48 shows a metaphase where numerous fragments, centric and acentric, several ring chromosomes, and long giant chromosomes are visible. Evidently in the preceding resting stage a wholesale breaks had occurred among the chromosomes. Many of these fracture surfaces had afterwards been again joined in sister or non-sister reunion (microphoto. 35). The large number of fragments showed, however, that such reunion had not occurred in all cases. An interesting chromosome, dicentric with difficulties of orientation, and produced by non-sister reunion, is shown in microphoto. 36. In this case the break must have occurred after the chromosome had been split up into its chromatids.

Darlington and Upcott (1941 b) have made a survey of hitherto known cases of spontaneous chromosome change. As is natural, almost all the cases are derived from botanical material. The causes of such breaks are very obscure, but are evidently of genetic nature, since they occur in connection with hybridity, etc. In the backcross hybrids Salmo trutta × (Salmo salar ×

× S. trutta), such spontaneous breaks with following non-sister reunion also occur, as previously mentioned. In the embryo of the small gwyniad the frequency of such breaks is evidently much higher, since in a single metaphase some twenty fragments, three ring chromosomes and at least three chromosomes of new type could be counted. Seeing that most breaks in all probability lead to restitution (*i. e.* reunion in the former condition) and therefore elude detection, the number of breaks in the preceding stage — though exact figures cannot, of course, be given —, must be extremely high.

This tendency towards the »breaking-up» of the chromosomes characterizes a large number of the nuclei of this embryo, as the metaphases show very different chromosome types. That all the acentric fragments and several other chromosomes — especially the giant chromosomes — do not survive more than a single mitosis is probable, and it is interesting to note that at metaphase they sometimes show signs of nucleic acid starvation, being more slender than normally. Also the metabolism of such a chromosome can evidently be disturbed, possibly by the loss of a heterochromatic part.

The meiosis presents nothing of interest, merely the usual technical difficulties. In one case, however, it was found, with a great degree of certainty, that 40 bivalents occurred in the first division of meiosis. No certain data regarding the possible occurrence of multivalents or univalents could be obtained.

11. The Grayling (Thymallus thymallus L).

This Salmonoid, which has not been cytologically studied by earlier investigators, likewise occurs in at least two, morphologically distinct forms or »subspecies». Only one of them, however, has been studied by the author. My material is derived from the Fishery Experimental Station at K ä l a r n e and comprises the northern form.

This species, viewed systematically, is rather remote from the *Salmo* and *Coregonus* genera. Like the smelt, it spawns in the spring. The development of the embryo proceeds much more rapidly in these two species, so that I had to fix my material at a still earlier stage, in order that the nuclei should not be too small. The grayling embryos were fixed after 36 hours. The chromosomes of the grayling are also rather different from those of the other species. This is manifested first and foremost in the number, which in the embryonal cells amounts to 102 — an unusually high figure (microphoto. 37).

The chromosomes are partly V-shaped, partly rod-shaped. They are very short, whence one cannot with complete certainty infer from their position the number of V-shaped ones. It seems that their small length — possibly

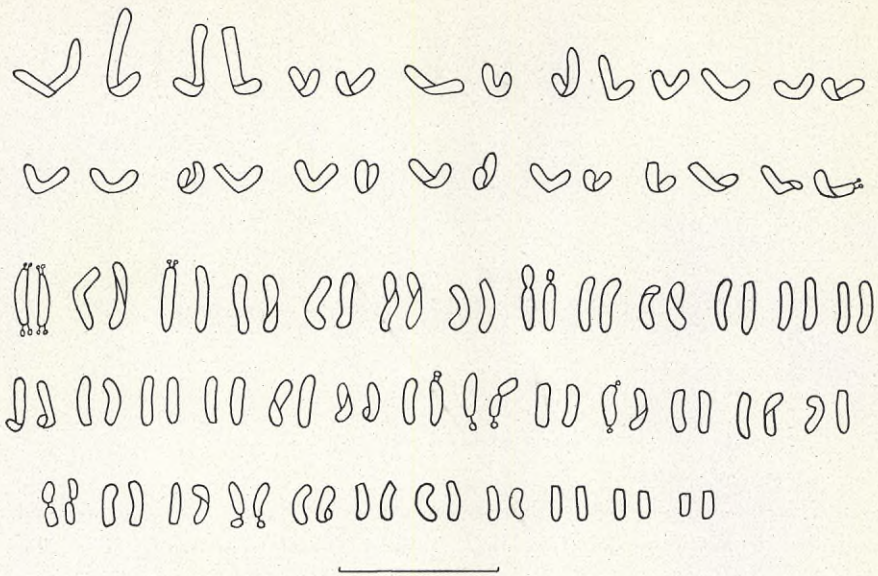


Fig. 22. Thymallus thymallus. Mitotic chromosomes.

owing to stiffness consequent on contraction —, prevents them from lying in the V-position which is so typical of the other species, with a well set-off apex. It is remarkable that it was not possible, even in good anaphase pictures, to obtain complete certainty regarding the number of V:s. The most probable number, however, is fourteen pairs. Nor can any exact data be given regarding the frequency of equal-armed and unequal-armed chromosomes.

Constrictions occur on at least one of the V-shaped pairs and on several of the rod-shaped. They may be either proximal or distal (text-fig. 22).

No more marked differences in size occur between the rod-shaped chromosomes, which form an evenly falling curve.

Studies of the meiosis have merely shown that the number of bivalents is about 51 and that in some cases univalents occur.

12. The Smelt (Osmerus eperlanus L).

The smelt has short and slender chromosomes, considerably smaller than in the other species. Their number is the lowest among the Swedish representatives of the Salmonidae family, their diploid number being merely 58 (microphoto. 38).

As a rule, however, both V-shaped and rod-shaped chromosomes occur. There are five pairs of V-shaped chromosomes. Two of them have arms of

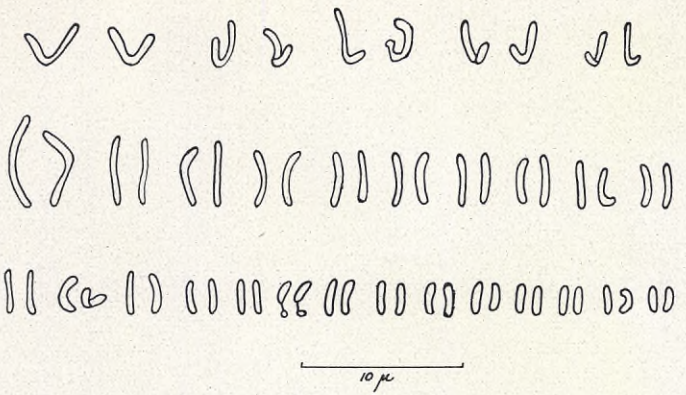


Fig. 23. Osmerus eperlanus. Mitotic chromosomes.

approximately equal length, whereas the other three pairs have one markedly long arm. Constrictions may occur distally in the short arm of one of these chromosome pairs.

The rod-shaped chromosomes vary in length. Thus the longest rod-shaped pair, at any rate in certain early metaphases, may be four times as long as the shortest. One of the rod-shaped pairs has proximal constrictions (text-fig. 23).

The meiosis was not studied.

C. Polyploid cells and embryos.

1. Polyploid cells.

It is well known, both from botanical and zoological material that isolated polyploid cells can be found in an otherwise purely diploid tissue. As a rule, however, discoveries of such cells — in animals — are made in testicles, where the spermatogonia in many cases may be tetraploid. Such isolated tetraploid nuclei have already been found in fish (WICKBOM 1943).

Also in the species examined by me isolated tetraploid nuclei are found now and then among the embryonal cells (microphoto. 39). This can scarcely be designated as remarkable or directly unexpected. On some few occasions I have also seen such tetraploid nuclei lying side by side with one another at full metaphase. This must signify either that the factor which has caused tetraploidy (somatic doubbling) has affected two ajacent nuclei simultaneously or else, which is more probable, that the tetraploid nucleus had divided

normally into two daughternuclei, each of which had now begun a new mitosis.

Unlike the conditions in the vegetable kingdom, no real polyploid tissues have as yet been produced in animals with the aid of colchicine (for the literature, see BÖÖK 1945). In view of this fact, BÖÖK has launched the hypothesis that polyploidy with the aid of colchicine, owing to the fact that the centrosomes are not paralyzed and consequently are multiplied during the c-mitosis (cf. LEVAN 1938, HAWKES 1942), must lead to the development of multipolar spindles and nuclei, which soon degenerate. The experiments hitherto made seem in fact to correspond with this, as pycnotic cells and malformations have been observed after colchicine treatment.

Polyploidy of a somatic nucleus must develop if the chromosomes pass through a division without the formation of any spindle. In animals the spindle must be organized by two centrosomes, but is also dependent on the cytoplasma. If the centrosome cleavage does not manifest itself or proceeds irregularly, so that more than two centrosomes are formed, the result will be either no spindle at all, a monaster, or a multipolar spindle. We now know that the chromosome division without the formation of spindles and without nuclear division, takes place, even on a large scale, in plants and animals. This important discovery has been made first and foremost by GEITLER (1937, 1938 a, 1938 b, 1939 a, 1940 a, 1940 b, 1940 c, 1941, 1944). Studies of this *endomitosis* have been made also by OKSALA (1939), GRAFL (1939, 1940), EKBLOM (1939), PAINTER (1940), ARARATIAN (1940), LEVAN (1944 a), and others. As typical of endomitosis, GEITLER (1938 a) states that the centrosomes are completely inactivated. The chromosomes during endomitosis may have a certain spiralization, though as a rule they are more or less unspiralized. An important matter is that endomitosis may alternate with ordinary mitosis.

It is obvious that the occurrence of tetraploid nuclei in an embryonal tissue, if it can be shown that they can pass through new mitoses, must imply either that they have been produced by endomitosis or that BÖÖK'S hypothesis cannot be correct, at any rate not completely so.

In order to understand the origin of the embryonal tetraploid nuclei, it is thus of importance that BÖÖK'S hypothesis should either be confirmed or shown to be incorrect. In order to arrive at such a decision, preliminary colchicine experiments were made. Eggs of *Coregonus* were laid in 0.5 % colchicine solution, where they were left for three hours, whereupon they were transferred to pure running water. The fixation was made after 9 hours.

Most of the embryos showed normal diploid nuclei in normal divisions. This was not particularly surprising, as previous experiments had shown

that it is difficult for colchicine to penetrate into the egg-shell, for which reason strong concentrations are required. Here and there, among normal nuclei some were found with a manifest colchicine effect. The chromosomes were contracted. Some of these nuclei were diploid, others tetraploid and some were in octoploidization, showing typical c-pairs (microphoto. 40). The effect of the colchicine was thus not uniform. No tetraploid anaphases however, could be observed. No conclusive evidence in disproof of BÖÖK'S hypothesis could thus be found.

The embryonal tetraploid nuclei may also be produced by endomitosis. A possible argument in favour of this interpretation is the fact that in one case a polyploidization — spontaneous — could be observed in the char (microphoto. 41). The chromosomes here divide into their chromatids. No anaphase movement occurs nor any metaphase orientation, which is an indication that no spindle had ever been developed. It is therefore conceivable that the centrosome cleavage may have failed. The chromosomes, however, are rather markedly spiralized, so that there can be no question of any *typical* endomitosis, but the phenomenon might nevertheless be regarded as such.

Such a spontaneous endomitosis in certain nuclei may be an abnormity, due to some hitherto unknown environmental factor. But it may also be a rare, but regular phenomenon, found in normal ontogeny. GEITLER (1944) indeed states (p. 183): »Das Kernwachstum (through endomitosis) erfolgt in den wachsenden Geweben *vor* ihrer endgültigen Fertigstellung und *nach* der embryonalen Vermehrungsphase». I consider it however to be quite conceivable that endomitosis may also occur normally in certain embryonal cells, for example in connection with their conversion into »organizers». If this were the case — seeing that the chromosomes show a normal mitotic contraction —, a natural transition in regard to spiralization, will be found up to the later typical endomitosis, which is a regular feature in the ontogeny. Further studies on this subject seem to be necessary.

2. Polyploid embryos.

In recent years several cases of spontaneous triploidy have been found among amphibia. In one case also a pentaploid embryo was found (for the literature see WICKBOM 1945; see also FANKHAUSER and HUMPHREY 1942). Such finds, however, have not been made in regard to fishes.

Among the Salmonoids spontaneous polyploid embryos and adult individuals seem to be rare. Among all the examined embryos of the pure species merely a single triploid embryo was found, namely of *Coregonus lavaretus*

from Älvkarleby 1944. This entire embryo consisted of cells containing 120 chromosomes. Likewise a spontaneous triploid embryo of the hybrid salmon × brown trout — which for the most part has an equally regular and undisturbed ontogeny as the pure species —, has been found. The chromosome number in this embryo was 100, which shows that — as might be presumed —, triploidy had been produced by the fertilization of an unreduced egg. The triploid embryo of gwyniad seems to have been produced in the same way.

A number of partial polyploids of *Salmo trutta* from Älvkarleby have been found in a consignment of roe. Ten carefully examined embryos showed the following chromosome numbers.

Embryo	Number of nuclei n	2n	3n	Unbalanced nuclei, chromosome numbers ± 1
1	2	156	—	—
2	6	133	—	—
3	61	5	11	20
4	56	7	13	17, 19, 20, 22
5	—	94	—	—
6	—	30	—	26, 26
7	1(?)	50	—	37, 19
8	—	64	—	—
9	1(?)	71	—	10, 14, 22, 24
10	11	67	—	23, 25

Nuclei with a sub-haploid chromosome number have several times been mentioned, and are thus nothing new. That the numbers are about 20 (n/2) has previously been noted in other connections. The cause of the disturbances, however, this time is unknown. Normally such nuclei do not occur in brown trout — but do occur in *Coregonus* —, and either the gametes represent some genetic unbalance, or else something has happened in connection with the artificial fertilization.

What is new in these embryos is that haploid and diploid nuclei may occur together. Where the haploid nuclei are numerous, triploid nuclei are also found (microphoto. 42—43). Embryo No. 3 has actually the chromosome numbers n/2, n, 2n and 3n. The query at embryos 7 and 9 merely signifies that there is some uncertainty as to whether the nucleus is haploid or unbalanced.

The haploid nuclei may be conceived to be derived either from a polar body which has been stimulated to develop, or else from other sperm nuclei.

Polyspermy is not normal in *Salmonidae,* but may be conceived to occur in exceptional cases. Moreover, it seems rather probable that, if the egg nucleus had not been reduced and had therefore given rise to a triploid embryo, other sperm nuclei may be specially stimulated to mitotic activity, as the triploid nuclei show a poorly developed mitotic activity. The combinations found can thus be explained, but there are many possible explanations. This is all the more evident as PARMENTER (1933, 1940) and KAWAMURA (1939 a and b) among *parthenogenetically* developed frog embryos found haploid, diploid, triploid and tetraploid embryos as well as those in which these numbers were intermixed, so that they were mosaic-like, as in my material.

The origin of the mosaic-like embryos, however, is partly explained by the cold treatment experiments (see p. 94).

D. Cold treatments.

In the course of the last ten years various experiments with cold treatments have been made in order to test the effect of different temperatures on the mitosis and meiosis. In these experiments interesting results have been obtained in several respects. Thus in the group *Amphibia* it was found that, if a newly fertilized egg was exposed to low temperature for some hours immediately after fertilization, a number of triploids and also haploids were produced (FANKHAUSER 1939, 1940, 1941 a, 1941 b, 1941 c, FANKHAUSER and GRIFFITHS 1939, FANKHAUSER and MOORE 1941 a, 1941 b, FANKHAUSER and HUMPHREY 1942, FANKHAUSER, CROTTA and PERROT 1942, GRIFFITHS 1941, BÖÖK 1940, 1941, 1943, 1945). These investigators had especially studied different species of the genus *Triturus.*

In *Triturus* the egg nucleus, when the egg has been fertilized and laid down, is in the second metaphase of meiosis. Although much labour has been bestowed on cytological investigations regarding the occurrence of polyspermy and the fate of the supernumerary sperm nuclei, no definite explanation has been given regarding the origin of haploid and triploid embryos. Seeing that they are produced at the same temperature, the meiotic stage of the egg nucleus when the cold shock sets in seems to be mainly responsible for the further development. Since a number of combinations may admittedly be produced in the parthenogenetic development of frog eggs (PARMENTER 1933, 1940, KAWAMURA 1939 a, 1939 b), the theoretically possible explanations are too numerous to permit any of them being regarded as highly probable.

It was of great interest to investigate whether such haploids and triploids

could be produced also in Salmonoid embryos. In addition, there were certain ascertained disturbances in the gwyniad and *Salmo trutta* embryos which might possibly be explained as due to a cold shock.

The first experiment with cold treatment was of a preliminary character. As great difficulties are involved by artificial fertilization in the laboratory, unless there is a supply of living breeding fish, the gwyniad roe was fertilized in the usual way in the lake W i a n, in B l e k i n g e, on the 10th November 1942 at 11.20. Ten minutes afterwards this fertilized roe was introduced into a thermos flask, at the bottom of which a piece of ice had been placed. Wood wool prevented the roe from coming into direct touch with the ice. The temperature in the flask could thus be expected to remain constant immediately above zero C. For various reasons the roe could not be taken out of the flask until 13.30 on the 12th November, when it was placed in a bowl with running water. The rise in temperature was equalized, so that it did not have the character of a shock. The gwyniad embryos were then kept in running water at a temperature of + 8 C. until dissection could be commenced on the 13th November in the evening and following night.

Cytological examination showed what had been expected after a morphological examination of the cleavage stages, namely that the normal cleavage mechanism was seriously disturbed in many of the embryos. Others, on the other hand, were apparently normal.

Thirty carefully examined embryos, fixed without selection, showed the following marked variations in regard to cytological data.

1. No mitoses at the time of fixing. The embryo probably had just died.
2. Altogether some sixty nuclei, the chromosome numbers of which could be determined more or less certainly. All distinct plates had exactly 40 chromosomes. Non-disjunction probable, as several other plates showed numbers from 36 to 43. This embryo was thus *purely haploid.*
3. Some eighty diploid nuclei. Variation round the number 80, however, considerable; good plates with 84 and 75 found. In addition, at least 5 nuclei with a haploid chromosome number and the following unbalanced numbers 52, 53 and 58.
4. Few mitoses. Some 20 diploid nuclei. In addition, 38, 42, 68, 71 and 76 found, all these numbers with an uncertainty of ± 2. Some additional nuclei had small, greatly contracted chromosomes with a marked tendency to clumping. The number of chromosomes in these nuclei very difficult to estimate, but probably about 50.
5. No diploid nucleus, but about one hundred haploid. Usual variation round 40, by one or two chromosomes. There are fragments, varying between 1 and 3, usually 1. This is evidently a pure *haploid embryo.*

6. No haploid, diploid or triploid nuclei. All the nuclei, about 50 at mitosis, have chromosome numbers of about 60 with an ascertained variation of 54—62, usually, however, 58—60. Fragments 0—2, usually one.
7. About 20 diploid plates. One nucleus with 34 may have been spread and thus not complete. The embryo probably *pure diploid.*
8. Few mitoses in bad positions. Probably *pure diploid.*
9. Markedly unbalanced embryo. Few mitoses, difficult to analyze owing to the fact that the chromosomes are short and narrow. Nucleic acid starvation. Approximate chromosome numbers 20, 19, 15, 11, 24 and 35. Fragments in several nuclei.
10. About 30 nuclei at mitosis. All triploid. Variation 115—122. A single chromosome bunch of 92 possibly broken, so that the number is uncertain. Fragments found. *Pure triploid.*
11. Only three mitoses with marked disturbances in spiralization, etc. Impossible to determine the chromosome number.
12. Few mitoses. Unbalanced embryo, with the following acertained numbers: 14, 15, 15, 16, 21, 22, 30 and 36.
13. A hundred nuclei at mitosis. All diploid. *Pure diploid.*
14. Many diploid nuclei, some with numbers under 80, in one case 75. *Pure diploid.*
15. Only two mitoses, in which, owing to marked disturbances, nucleic acid starvation, spiralization (?), it was impossible to determine the chromosome number with any degree of certainty.
16. Few mitoses. Some thirty nuclei with high chromosome numbers, probably about 100. One nucleus doubled — about 200. A few nuclei with other numbers, a good metaphase with 56. Nucleic acid starvation, the chromosomes not distinct, diffuse in contour; possibly uncoiled. Anaphase sticking occurs.
17. Markedly unbalanced embryo, but many mitoses. One part of the embryo consists of nuclei with 33 chromosomes (some 10 mitoses), another part, on the other hand, has a chromosome number of 108 and one fragment. The numbers 45, 84, 90 and about 100 have been noted. Chromosome elimination occurred in some cases.
18. About 30 metaphases are diploid, some haploid (5 observed) and a considerable number of nuclei with 11 chromosomes. Some 40 of them were at metaphase. In addition, in individual cells: 30, 23, or 24, 25, 27 (2 distinct metaphases) and 46.
19. Some 60 metaphases of diploid type, but all the controlled plates showed

76 or 77 chromosomes. In addition, individual cells with 31, 34, 36 and about 145 chromosomes.

20. Only 5 diploid nuclei. Some 70 nuclei with high chromosome numbers, half of them with about 100, the remainder with about 120 chromosomes, thus *triploid* tissue.
21. A particularly interesting embryo without marked chromosome variations. About 100 mitoses observed; in all the controlled cases the chromosome number was 35, 36 or 37. This embryo was thus homogeneous with a chromosome number near the haploid. The chromosomes entered the metaphase plate with distinct constrictions, thus but little spiralized. Anaphase sticking occurred rather frequently.
22. Very unbalanced embryo with distinct mitotic disturbances. Anaphase sticking and a general tendency in the chromosomes to become pycnotic. Chromosome elimination occurred, as well as defective orientation in time. Owing to the disturbances, the chromosome numbers could only be roughly estimated. Some nuclei with numbers about 70, but most of them about 20. The smallest number observed was 15.
23. More than half of the embryo haploid, with one fragment. In addition, other numbers occurred: 53, 44, 16, 15, 13 and 6, of which four distinct metaphases were studied. Several nuclei pycnotic with poorly spiralized chromosomes.
24. *Pure diploid*, many mitoses.
25. *Pure diploid*, many mitoses.
26. About one-third of the embryo diploid, but with variation (78 and 76 counted). Otherwise many other chromosome numbers, each represented merely by single nuclei: 35, 58 with fragments, 64, 30, 34, 64, 66, 32, 31, 47 with several fragments, probably the result of diminution. Anaphase sticking observed.
27. Entirely unbalanced embryo. Defective orientation in the metaphase plate and irregular chromatid cleavage observed. Chromosome numbers: many good metaphases with 28, about 118, 22, 10, 40, 47 with fragments, 16, 20, 37, 110, 27, 17, 34, 89, about 110, 30, 8, 92, 25 and 7. As regards the types 7, 8 and 10, several nuclei of each occurred.
28. Entirely unbalanced embryo. Distinct metaphase plates without disturbances. The following numbers were observed: 102, 63, 60, 58, 37, 36, 35, 11, 9 and 4 (see microphoto. 47). The chromosome number 4 is the lowest found, and even these nuclei were capable of mitosis, which was indicated by the fact that anaphases were observed and that the nuclei with that number were several in one group.

29. About a hundred nuclei with the triploid chromosome number 120. *Pure triploid.*
30. More than a hundred mitoses, without disturbances, all diploid. *Pure diploid.*

These thirty cold-treated gwyniad embryos give us a good idea of the abnormal chromosome conditions which may arise in such treatment (microphoto. 44—47).

The results obtained in *Amphibia,* with a production of balanced haploids and triploids, have been shown to be applicable also to *Pisces,* in that two haploid and two triploid embryos were observed. Despite the considerable duration of the cold treatment, 7 pure diploids, which had thus escaped being affected by the treatment, were also found. This must be designated as very remarkable.

By far the great majority of embryos, however, show greatly varying chromosome numbers and are thus »mosaics», as has been previously shown in the case of genetic disturbances. This result of cold treatment was then unknown, but at about the same time BÖÖK (1943, 1945) found that *multiform aneuploidy,* as he calls it, is regularly the result also in *Triturus,* if the treatment is started later than thirty minutes after insemination.

Among the embryos which show multiform aneuploidy, there are several categories of special interest. Two of them are partially diploid, which seems to mean that the disturbances caused by the cold treatment had affected the egg after the cleavage had in part already commenced, so that a diploid zone remained unaffected. One embryo is partly haploid, whence it might have been included among the pure haploids if the treatment had terminated at an earlier stage. Two additional embryos had both haploid and diploid zones, in addition to the unbalanced one, thus completely resembling the previously mentioned embryos of *Salmo trutta* (p. 85). One embryo is diplo-triploid with a zone of about 100 chromosomes, *i. e.* the intermediate number. Also in another unbalanced embryo there is a zone with 100 chromosomes. Two embryos, besides the usual variation owing to non-disjunction, are homogeneous, with chromosome numbers about 60 and 35—37, thus near the haploid number.

Nuclei with 10, 8, 7, 6 and even 4 chromosomes have been found capable of passing through mitoses, even several mitoses in succession, without appreciable disturbances.

As a further result of this preliminary experiment, two embryos of the haploids and triploids were found, one with and one without a fragment,

which is a matter of importance in determining the question as to which sex is the heterogametic.

However, among the mitotic disturbances, which are of essentially the same type as in the back-cross hybrids *Salmo trutta* × *(Salmo salar* × *S. irutta),* no certain case of multipolar spindles has been observed, which is of the greatest importance.

It seems to be beyond doubt that haploids, triploids and the embryos which show a partial or entire multiform aneuploidy have been produced by the effect of cold on the spindle. That the spindle is very sensitive to chemical and physical agents is now well-known (DARLINGTON 1937, CALLAN 1942, CALLAN and BARBER 1942, LEVAN 1938, FANKHAUSER, a number of works, TISCHLER 1942, 1943, ÖSTERGREN 1944 a, BÖÖK 1945, WICKBOM 1945, and others). If the regular course of the egg's meiotic divisions is disturbed by inefficient spindles, haploids and polyploids will develop and also multiform aneuploids; on the other hand, if the embryonal somatic mitoses are disturbed, partial multiform aneuploidy will be produced. Hence, the fact that no multipolar spindles are observed in the fixed gwyniad embryos as well as the relative absence of spindle defects in general, indicate that nucleic acid starvation with anaphase sticking and other mitotic disturbances had occurred secondarily after the cold shock had produced the first nuclei with chromosome numbers deviating from the normal.

In order to explain the origin of balanced haploids and triploids of *Triturus* embryos after cold treatment, BÖÖK (1945) has set up the following hypothesis, based on the supposition that the low temperature prevents normal spindle development.

1. If the temperature shock affects the egg immediately after fertilization, when the egg is in the second metaphase of meiosis, anaphase movement is prevented by the blocking (destruction) of the spindle. Diploid restitution nucleus with two centrosomes is then formed. The sperm nucleus with its centrosome fertilizes this nucleus and the two centrosomes of the egg are eliminated in the normal, but hitherto not fully explained, way. Conditions have thus been created for the development of a balanced triploid.
2. If the cold shock affects the egg somewhat later, *e. g.* when it is in the second anaphase, a similar paralysis of the spindle occurs. The chromosomes remain in the anaphase position without being able rapidly to reorganize a resting nucleus. When the temperature rises, the sperm nucleus is again activated; the anaphase configuration has not such a power of attraction on the sperm nucleus, and the result is that the

centrosome of the sperm nucleus divides into two, thus beginning a mitosis. In this way a haploid embryo has started its development. The chromosome set is thus *paternal*.

3. If the two nuclei are at rest and no spindle has developed, the low temperature will merely defer the coming mitosis owing to the generally reduced rate of development. Thus when the temperature is again normal, the embryo can continue its normal development, which results in diploidy.

For a definitive settlement of these questions, sectioning of the treated material seems to be necessary. In the case of eggs rich in yolk, however, such an investigation will meet with great technical difficulties, as is also pointed out by BÖÖK. This in fact seems to be the reason why FANKHAUSER and his school have not yet been able to give a definitive explanation. There is, however, also another expedient, namely to use a hybrid between two species which have chromosomes that can be distinguished either in form or number. Such hybrids, however, as a rule are not so undisturbed in their normal development that differences from pure species cannot be presumed to have arisen from hybridization.

Fortunately, however, *Salmo salar* and *Salmo trutta* form an extremely favourable exceptional case. They have distinctly different chromosome numbers and the hybrid between them, during the first stages in question, shows no noticeable differences as compared with the corresponding development in the pure species. If salmon eggs are fertilized with brown trout milt and the hybrid is subjected to cold treatment, the following categories should be distinguishable among the embryos.

1. Normal diploids, produced after fertilisation (70 chromosomes).
2. Apparent diploids, produced by duplication of the egg or sperm nucleus (60 and 80).
3. Haploids. Two types can be distinguished, maternal with 30 and paternal with 40.
4. Triploids of four different kinds with the chromosome numbers 90, 100, 110 and 120.

In the autumn of 1944 I made cold treatment experiments on this hybrid. The results are shown in the subjoined table.

Owing to technical difficulties the temperature, which was kept constant with melting ice, was not so low as would have been desirable. In the course of the experiment it varied between 0.3—1.2 degrees centigrade.

Table 4.

After fertilization, minutes . .	10	10	15	30	30	60	60	120	120	0
Duration of the cold shock in minutes	60	180	360	60	180	60	180	60	180	0
Number of embryos with 70 chromosomes	13	10	30	18	12	11	17	11	17	25
Number of embryos with 60 chromosomes	—	—	2	—	—	—	—	—	—	—
Number of embryos with 30 chromosomes	—	1	2	—	—	—	—	—	—	—
Number of embryos with 100 chromosomes	—	—	—	—	—	—	—	1	—	—
Number of embryos with 70 chromosomes with a few interspersed 40-chromosome nuclei	2	1	—	—	—	1	—	—	1	—
Number of embryos with multiform aneuploidy	1	2	6	—	—	1	—	—	1	—
Total embryos	16	14	40	18	12	13	17	12	19	25

This seems to be the reason why so few haploids and triploids and so few cases of multiform aneuploidy had occurred. The experiment, however, enables us to draw certain conclusions of interest. They are the following.

1. The majority of the embryos showed the chromosome number 70, produced by the fertilization of a haploid egg nucleus of *Salmo salar* (30) with a haploid sperm nucleus from *Salmo trutta* (40). This shows, in conjunction with the appearance of the controls and previous experience, that the hybrid normally does not exhibit any disturbances due to hybridity.
2. Two embryos showed the chromosome number 60, produced by restitution in the meiosis of the egg nucleus or later. No fertilization had taken place, the embryos were therefore *parthenogenetic.*
3. Three haploids with the chromosome number 30 clearly show — by the absence of haploids with 40 chromosomes —, that haploids are produced parthenogenetically by the mitotic activity of the egg nucleus. The haploids produced in the cold treatment of salmons are thus in all probability *maternal* and not, according to BÖÖK's hypothesis, paternal.
4. One triploid, as well as a previously reported spontaneous appearance, was produced by the fertilization of an unreduced egg by the haploid sperm nucleus.

5. An unexpected group of embryos are the five which in a normal diploid tissue (70) have a few interspersed nuclei with the haploid number from the *male* (40). Seeing that fertilization had evidently occurred, the sperm nucleus must either have divided once before fertilization, or else polyspermy had occurred, so that a second sperm nucleus had begun to divide, whereupon its progeny were included in the normal embryonal, diploid tissue. Polyspermy — in contradistinction from the conditions in amphibia —, is known only in exceptional cases as regards the fishes (MOENKHAUS 1904; cf HUETTNER 1927 on Drosophila). Nevertheless polyspermy in this case seems to be a more probable explanation than that the sperm nucleus had passed through a mitosis before fertilization.
On the assumption of polyspermy, we can give a plausible explanation of the embryos of brown trout (mentioned p. 85) which showed haploid nuclei in diploid or triploid tissue.

As the conclusions in points 2 and 3 imply that the haploids of gwyniad produced by cold treatment were maternal, this signifies, seeing that in the previously mentioned cold treatment one of two haploids had a fragment, whilst the other was devoid of it, that in the gwyniad the female must be heterogametic (cf. the discussion on p. 77).

In the Salmonoids the egg nucleus after fertilization is probably in the first meiotic metaphase (BÖHM 1891), although variations may conceivably occur in artificial fertilization, when the female is compelled to lay her eggs, whereas in *Amphibia* the meiosis has proceeded further, namely to the second metaphase. This may possibly involve differences in the production of haploids, but I consider it probable that the conditions are essentially similar and that the haploids, also in *Amphibia*, are produced in cold treatments by stimulating the reduced egg nucleus to divide.

E. Mitotic genes and their bearing on the interspecific sterility-barrier.

Mitotic disturbances of various kinds have been reported and subjected to detailed description several times in preceding pages. It is a matter of importance to attempt a closer analysis of the causes of these disturbances.

Mitotic disturbances have been ascertained in the following cases:

1. Spontaneously, in a certain frequency of gwyniad embryos. Evidently, a normal occurrence.

2. Spontaneously, in brown trout embryos. Possibly, due to external influence.
3. Regularly, in the hybrids salmon × brown trout, back-crossed to brown trout.
4. In cold treatments.

These mitotic disturbances have varied in strength with, however, distinct mutual characteristics. The normal nucleic acid cycle of the chromosome has been deranged. This has involved a typical anaphase-sticking and varying stainability of the chromosome and its respective parts. The co-operation between the contraction-cycle of the chromosome and the spindle has been interrupted in both directions, giving rise to orientation difficulties as well. The centrosomes have been affected, either directly or indirectly, causing the appearance of multipolar spindles. Further, the stability of the chromosomes has decreased, producing instead a strongly increased frequency of chromosome mutations.

Since the disturbances in the gwyniad embryos occur in a certain frequency of similarly treated but otherwise normal embryos, the explanation of the observed mitotic disturbances is, in all probability, to be found in the fact that, owing to irregular meiosis, gametes have developed with an unbalanced chromosome set. Accordingly, the cause of the disturbance is a genetic lack of balance. The same applies to the back-cross hybrids.

The disturbances in the brown trout embryos may be due to an involuntary cold shock undergone in connection with the artificial fertilization. However, the disturbances produced by the cold treatment may either be primary or secondary. The spindle is known to become damaged by cold treatment. This gives rise to the appearance of nuclei with varying chromosome numbers. The embryos subjected to cold treatment have, nevertheless, been examined when a considerable time has elapsed after the discontinuation of the cold shock itself, and several new nuclear generations have passed. Accordingly, the disturbances remaining at the time of the fixation procedure may, quite likely, be secondary ones, *i. e.* due to the genetic incapacity of the nuclei with separate, unbalanced chromosome numbers to accomplish normal mitoses. Since we are, as yet, unaware of any definite cold effect other than that of the spindle disturbances and nucleic acid changes, the changes noted at the cold treatments have probably been chiefly secondary, *i. e.* of a genetic origin. Thus, a very strong probability speaks in favour of the contention, either that the mitotic disturbances depend on the absence in the nucleus of the genes required for the occurrence of a normal mitosis, or that their normal co-operation has been interrupted. There

is, therefore, good reason for employing the term *mitotic genes* in order to indicate genes required for the accomplishment of normal mitosis.

It goes without saying that these genes must be of considerable importance, since ontogenetic development is inconceivable without normal mitoses. However, our knowledge of these mitotic genes is exceedingly deficient. It is, above all, through the genetic changes that we can obtain any knowledge of them, and mutations or re-arrangements of the mitotic genes will often lead to quick death. This is probably the reason for TISCHLER'S (1942) otherwise incomprehensible statement (p. 339) regarding the causes of the mitotic disturbances, *viz.*, »Gegenüber den Aussenfaktoren spielen die inneren 'genetischen' Faktoren anscheinend eine sehr geringe Rolle».

Considering that many and, in all likelihood, the great majority of the properties of an individual are controlled by polygenes (valuable theoretical conclusions regarding these polygenes have been drawn by MATHER 1941 and 1942, SISMANIDIS 1942, and HASKELL 1943), it may easily be supposed that the mitotic genes should be numerous and distributed among all the chromosomes of the genome. Therefore, it may seem inexplicable that certain nuclei in the Salmonoids can be proved capable of performing mitoses with a strongly reduced chromosome number. In the gwyniads, nuclei with only 4 chromosomes, *i. e.* a tenth part of the haploid quantity, have been ascertained as capable of mitosis, though not without some slight disturbances. This important fact can, apparently, only be explained by means of the following alternative assumptions:

a) The mitosis is not subjected chiefly to genetic control, but is mainly directed by the cytoplasma.
b) Nuclei with normal chromosome sets are capable of controlling the mitoses of other adjacent nuclei which lack a normal chromosome set.
c) The mitotic genes are concentrated to a few chromosomes, and each chromosome may, possibly, have a set of genes permitting the mitotic division.
d) The present chromosome numbers of the Salmonoids form multiples of a lower basic number, *i. e.* the Salmonoids are polypoids.

Assumption a) is, for several reasons, extremely improbable. Perhaps, the back-cross hybrids are the most convincing ones. In their case the cytoplasma is that of *Salmo trutta,* and it is the sperms that cause the varying chromosome numbers and, consequently, also the lack of genetic balance. If no special mitotic genes exist, there does not seem to be any reason why these hybrids should differ from the normal brown trout embryos which do not disclose any disturbances.

Nor is assumption b) particularly likely. We know, of course, as yet very little about the various fields of activity of the genes, nor can we say whether or not one nucleus is capable of controlling the functions of another. However, at cold treatment whole embryos have been obtained where not a single nucleus had the normal chromosome number, and in one instance even lacking the haploid quantity. When a whole gwyniad embryo, comprising hundreds of nuclei, has a chromosome quantity varying from 35 to 37, there does not seem to be any possibility of conceiving a controlling impulse from the normal nuclei.

On the other hand, assumption c) cannot be easily repudiated. The mitotic genes may either be restricted to a few chromosomes, or else each chromosome may have a set of mitotic genes. However, it is unlikely that the mitotic genes are concentrated to a certain small number of chromosomes. Proceeding from the established lowest number of chromosomes in gwyniads necessary to produce mitosis, *viz.*, 4 chromosomes (in salmon 6 chromosomes, unpublished), a series of higher chromosome numbers should permit mitotic division without any difficulties. Still, this is not the case, as proved by the results mentioned earlier. The probability that these four »mitosis-chromosomes» occur among the chromosome numbers 70—80 in gwyniads is very great, but nevertheless disturbances are frequent in this connection. It is far more probable — and moreover in greater confirmity with our present conception of the polygenes — that each chromosome in a set may be capable of organizing a mitosis owing to the fact that the mitotic genes are divided into groups, each group constituting a more or less functional unity.

This may appear too daring a conclusion and stands in opposition, *inter alia,* to the definition of a *set* itself, as may be seen from the following quotation from DARLINGTON and THOMAS (1942, p. 127): »The ordinary chromosomes making up the haploid set of an individual or a species are all necessary for the regular development of every tissue and of nearly every isolated cell. Indeed, that is how we have to define a *set* of chromosomes.» The question now arises whether the present haploid numbers in the Salmonoids are also original ones, or has a multiplication in the phylogeny taken place?

Assumption d) derives strong support from a series of other facts regarding the chromosome conditions of the Salmonoids which will be reported in detail below. It shall only be pointed out here that the mitotic disturbances appear to be particularly strongly bound to certain chromosome combinations, with the result that other chromosome numbers, on the other hand, show a better balance. It has been stated several times that the nuclei with 20 chromosomes, or thereabouts, seem to occur more

often than pure chance would admit. Unfortunately, however, it has not been possible to carry out a statistical analysis owing to the uncertainty in the determinations of the chromosome numbers in several disturbed mitoses. Thus, gwyniad embryos with 20 chromosomes, brown trout embryos with the same quantity, and a special chromosome number in gwyniads after cold treatment of approximately 60 are examples strongly favouring the assumption of an inner balance. This balance would then manifest itself, above all, in the fact that nuclei with chromosome numbers of about 20, 40, 60, 80 and 100 have a mitotic capacity exceeding, on an average, that of other nuclei. Together with the many other, in part, still more marked indications (cf. Chapter on Chromosome phylogeny), the mitotic balance as regards the Salmonoids seems to imply that these fishes are actually polypoids. A hypothetical basic number would then be 10, possibly even 5, which is near minimum number for a mitosis.

These facts are of decisive importance with regard to an understanding of the mitosis disturbances in the Salmonoids. The mitotic genes may, then, apparently be distributed in an originally haploid set and DARLINGTON'S and THOMAS' definition of the set should, accordingly, be correct. However, the possibility that each chromosome possesses a sufficient number of mitotic genes cannot, it seems, be dismissed as directly improbable. Sc far, information is admittedly scant regarding the nuclear capacity for life with a reduced gene number in plants (MC CLINTOCK 1941), while, in the animal kingdom, several data have been presented of a marked chromosome variation in the soma (SHIWAGO, GOLDRIN and WOLOCHOW 1937, TSCHERNOSHUKOWA 1939, HEBERER 1940, PLETNEW 1941 and MATTHEY 1941). Future research will reveal the extent to which polyploidy may play a part in this connection.

The mitotic genes appear, for natural reasons, to be of extreme significance with regard to the formation of the sterility barrier between the various species which is of such great evolutionary value. As already mentioned (TISCHLER 1942), the mitotic disturbances in hybrid plants are not particularly common. The plants have an interesting form of sterility, called somatoplastic sterility (COOPER and BRINK 1940), where the death of the embryo is, in the first place, due to a deficiency in the nutritional supply caused by the inadequate development of the endosperm. Irregular mitoses have *not* been observed. On the other hand, in animals matters are different. PAULA HERTWIG (1936) reports in her textbook on animal hybrids, long series of cases where pronounced mitotic disturbances have been noticed.

Thus, it is a general experience that in hybridized animals — which are

not closely related to one another — the fertilized egg runs through a series of mitoses with marked disturbances which rapidly lead to the death of the embryo. Multipolar anaphases with chromosome elimination have been observed very frequently. The older zoologists have explained this as due to the incapacity of the paternal chromosomes to work in the normal way in foreign cytoplasma. In the majority of cases, however, a more adequate explanation will be found in the difference in strength and balance between the mitotic genes of the two species which is too marked for them to accomplish normal mitoses, when they are brought together.

P. HERTWIG (1936) also mentions several cases with more or less definite signs of elimination of the paternal chromosomes. In many cases, no doubt, definite proof does not exist but, on the other hand, it has been clearly established that the chromosome elimination may involve certain definite chromosomes (BERRY 1941, REITBERGER 1940). This cannot apparently be otherwise accounted for than by the fact that the chromosomes have a certain degree of autonomy (cf. CORNMAN 1944). CORNMAN states, *inter alia*, that »chromosome elimination occurs in others of the fungus flies (apart from Sciara) and autonomy is not restricted to special organisms» (p. 412). This conception of the chromosomes must, amongst other things, involve a change in our opinion of the centromeres since they do not seem so uniform as assumed earlier by, for instance, the DARLINGTON school. Obviously, the centromeres play an important part in chromosome elimination, constituting as they do the movement centre of the chromosomes. Thus, the conception of the autonomy of the chromosomes greatly facilitates an understanding of the appearance of more or less balanced chromosome numbers round certain multiples, such as in the Salmonoids.

The part played by the mitotic genes in crossed species is noteworthy even in crosses between animals which are not so widely different and where an embryonic development actually takes place. The hybridization of *Salmo trutta* × *Coregonus lavaretus* discloses an exceedingly variable development of the embryos which has been subjected to morphological examination (RUBASCHEV 1935). Notwithstanding the fact that, so far, cytological studies of this hybrid are lacking, it seems very likely that this disturbed embryonic development is in the end dependent on mitotic changes. F1-hybrids between brown trout and char (SVÄRDSON, unpublished) show certain characteristic and less marked disturbances, such as the loss of the dorsal and adipose fins, bends in the spine, etc. This may, in all likelihood, be explained by the incomplete chromosome number obtained by certain nuclei which has later, whether directly, or owing to the organization of embryonic development by the nucleus, involved a disturbance in the morphological progress.

When the species employed in hybridization are closely related, as, *e. g.*, salmon and brown trout, the mitotic genes seem to co-operate well in the hybrid, giving rise to quite normal development. When this hybrid has undergone meiosis, where segregation disturbances occur owing to the lack of chromosome homology between the species, gametes are formed with varying chromosome numbers. These gametes will then in F2 probably cause typical mitotic disturbances, as mentioned above concerning the back-cross hybrids.

Thus, the significance of the mitotic genes with regard to the sterility-barrier between different species is great and the genes can exert their activity in F1 as well as in F2. It is hard to decide the extent of their influence in hybridizations between systematically widely different species. In this connection the question of the part played by the cytoplasma has again to be taken into account. KAUFMANN (1940) observed that eggs of the F1-hybrid *Drosophila miranda* × *D. pseudoobscura,* race A, at fertilization of sperms from either of the main species produced embryos which soon died after a few extremely abnormal mitoses, where multipolar spindles, etc., were noted. He interprets this as the result of a maternal effect and states (p. 208): »The hybrid chromosome complement has so conditioned the egg cytoplasma prior to fertilization that meiotic disturbances, irregular cleavages, and abnormal polar chromosome multiplication may take place». Personally, I am of the opinion that the mitotic genes are unable to co-operate in this case and that this is the cause of the disturbance.

Undoubtedly, the part played by the cytoplasma at the hybridization of species cannot after MICHAELIS' investigations of *Epilobium hirsutum* be denied (see, *e. g.*, MICHAELIS and V. DELLINGSHAUSEN 1942). However, it remains as yet to be proved whether or not *Epilobium* is a special case. Generally speaking, certain useful conclusions may be drawn from these investigations. This applies, above all, to the genetic reaction chains and the delimiting influence of the cytoplasma in this respect. In certain cases, the effect of the genes may in this way be clearly inferior to that the cytoplasma. Moreover, it seems undeniable that the genetic activity involves an organization and activation of the cytoplasma and the, perhaps, varying chemical composition of the plasma must be attributed some significance with regard to the activity proper of the gene. Thus, there is an intimate co-operation between, *e. g.*, mitotic genes and the cytoplasma. However, we should not, in my opinion, by using inadequate terms unnecessarily add to the difficulty of understanding these intricate processes. The definition »cytoplasmic influences» would, therefore, in several instances be less correct than that of »activity of the mitotic genes».

V. General discussion of Salmonoid chromosome phylogeny

In the preceding pages, an account has been given of the results of an examination of the chromosomes occurring in a number of Swedish Salmonoids. As already pointed out in the introduction, my main purpose has been to ascertain whether any general conclusions regarding the chromosome phylogeny of these species can be drawn from these studies. In the presentation of the chromosome sets, etc., of the different species, the problems directly connected with the chromosome phylogeny has not been discussed and will be covered separately in a special discussion below.

A. Chromosome morphology and number.

The older cytologists thought that by concentrating on studies of the chromosome morphology and chromosome number in a series of species they could establish a phylogenetic interrelationship between them. The mutual relationship between two species may be said to have been fully established through knowledge of all their genes and the position of these genes in the chromosomes. However, our knowledge will never reach such a point, though it must be admitted that the *Drosophila* research is well on its way.

In later years, when our knowledge of the nature of evolution has been considerably added to, several authors have drawn attention to the fact that gene mutations and not sectional re-arrangements within the chromosomes constitute the principal raw material of evolution, alongside of crossing-over and segregation (MULLER 1941, HUXLEY 1942, BAUER and TIMOFEEFF-RESSOVSKY 1943, among others). When a series of species has a monophyletic origin, then the part played by the genetic changes has been greater than that of the structural changes within the chromosome set. However, there is still a possibility that »gene mutations» are, in actual fact, exceedingly small re-arrangements close to or within a gene, and that they might be interpreted as structural changes (cf. MULLER 1941, who subjects this problem to detailed analysis).

From this principle of evolution of the chromosomes it follows, as a matter of course, that the external morphology of the chromosomes, being more

unchangeable than the gene mass, will remain to some extent as a characteristic of a group of related organisms a considerable time after the development of the genetic divergency into the order of a species.

Moreover, as a general rule, experience has taught that a group of related species will most often disclose, morphologically, quite similar chromosomes. DARLINGTON (1941 b) cautiously states that the chromosomes are not »uniformly conservative in different groups but we sometimes find in them the means of recognizing the common descent of groups of species and even of genera and families» (p. 155). However, the exceptions from this rule are not exactly infrequent as shown in a few instances by DARLINGTON *(loc. cit.)*.

These exceptions where the chromosome morphology changes during the process of evolution are of great interest. During later years, several investigations have been carried out with a view to throwing light on the mechanism of this change. Now we are able to state that we are in the possession of that knowledge.

Changes in the chromosome morphology may be of two kinds, *viz.*, changes in the shape of one or a few chromosomes without involving a change in the number, and changes affecting the chromosome number.

Morphological changes without a simultaneous change in the chromosome number occur when, for some reason or other, the chromosomes break in one or several points, after which the chromosome pieces immediately become reunited, though in a different order. Nowadays, a distinction is made as between deletion, duplication, translocation, segmental interchange and inversion. Only a few years ago, such structural changes were believed to be extremely common in natural populations. Recent research has, however, shown that the extent of their appearance varies fairly greatly, inversions generally being the most frequent with, perhaps, the exception of duplications (DOBZHANSKY 1939 a, DOBZHANSKY and SOCOLOV 1939, GEITLER 1939 b, SOKOLOV and DUBININ 1940, SESHACHAR 1939, LÖVE 1944, and others). The reason why the structural changes are not so easily incorporated in a population is, above all, the sterility of the heterozygotes (cf. MULLER'S, 1941, detailed discussion of this problem, and HUXLEY 1942). Thus, the diffusion obstacles existing especially with regard to the more extensive structural changes will serve as a natural explanation of the conspicuous constancy often manifested in the chromosome morphology.

Variations in the chromosome number have been observed repeatedly in related species. Two rod-shaped chromosomes in one species are found to correspond particularly often to a V-shaped chromosome in another species, causing the haploid chromosome number to be reduced by one. This phenomenon has already been observed by earlier cytologists and is called the Robertson law

(Mc Clung 1914, Metz 1914, 1916 and Robertson 1916). The validity of this law has been demonstrated with regard to the *Reptilia* by Matthey (1931, 1933, 1939), the *Amphibia* by Wickbom (1945), the *Diptera* by Wolf (1941), and by Sturtevant and Novitski (1941) in their comprehensive survey of the *Drosophila* literature. As regards botanical material, this phenomenon has been referred to, *i. a.*, by Levan and Emsweller (1938), Cave and Bradley (1943), Togby (1943) and Garber (1944).

Accordingly, this phenomenon is well known from experience, though its explanation was not easy to trace. The older cytologists assumed simply that the chromosome split at the centromere and its parts became independent chromosomes. One of the last authors to steer clear of this as yet undefined obstacle was Vandel (1937) who writes, as follows (p. 519): »It has been known for a long time that the fragmentation of chromosomes into shorter elements, or inversely, the fusion of simple chromosomes into compound chromosomes, is a frequent phenomenon in certain groups and allows one to give an account of the variety of chromosome numbers that can be seen in neighbouring species. The break of the chromosomes occurs at a fixed point of least resistance generally corresponding to the point of insertion of the spindle fibres. For instance, a V-shaped chromosome splits itself into two fragments, each taking the shape of a batonnet ... But all this is well known and there is no need to linger on it.«

In fact, at the very time when Vandel was writing this, a number of scientists had long been devoting attention to this problem, for »the difficulty of formation of two 'rods' from one V arises from the fact that, whereas the one V has but one centromere and two telomeres, the two 'rods', considered together, have two centromeres and four telomeres« (Muller 1941). Thus, in the event of a chromosome increase, in accordance with the Robertson law, a centromere must be new created in some way. This constitutes the actual problem here. It is, of course, possible for the chromosomes to reach the anaphase pole in mitosis without centromere, though they are then considerably delayed. However, it is ejected without fail in the next nuclear generation (Carlson 1938, confirmed by a number of earlier, as well as more recent studies). It is only in the *Hemiptera* that chromosomes or fragments without a centromere reveal a capacity of survival, their centromere being of a so-called diffuse type which has been far from elucidated (Hughes-Schrader and Ris 1941).

A possibility of explaining the occurrence of V's and rods by translocation was suggested by several authors at an early stage. Navashin (1932) supposed that both the arms of the V were translocated over to other chromosomes

and the loss of the centromere would then make no difference. This applied to chromosome reduction. Conversely, an increase in the chromosome number could be produced when a centromere, surrounded by short heterochromatic (inert) arms, could by means of irregular division become supernumerary. Other chromosome arms could then, secondarily, be translocated over to this almost »loose» centromere. The inert Y-chromosome of the *Drosophila* was specially pointed out as a centromere-giver of this kind. (MULLER and PAINTER 1932). DUBININ (1934) proved that this process was realizable by experimentally changing (*i. e.* reducing) the *Drosophila* chromosomes via the centromere of the Y-chromosome. This conception of the changes in the chromosome numbers via translocations was shared by, *e. g.*, BABCOCK and CAMERON (1934) and STURTEVANT and TAN (1937).

The weak point in these hypotheses was that they presupposed a complicated succession of breakages and reunions prior to the occurrence of a stable change. Therefore, useful theoretical help was obtained when the frequency of breakages was found to be greatest in the heterochromatin, which is often situated just close to the centromere, thus facilitating a translocation of whole arms (BAUER 1939, GILES 1939, SAX and MATHER 1939, PROKOFIEVA-BELGOVSKAJA and KHVOSTOVA 1939, GILES 1941, HELFER 1941, BELGOVSKY and PROKOFIEVA-BELGOVSKAJA 1943).

Nevertheless, it is admittedly rather hard to conceive a more extensive rearrangement of the chromosome arms by means of translocation from V's to rods, and *vice versa*. MULLER (1941) makes the following remark in this connection: »This type of change could be expected to arise only on very rare occasions» (p. 222). DARLINGTON (1940 b, p. 359) pursues the same train of thought in saying that »the indications of fragmentation and fusion at the centromere ... have been difficult to understand on the basis of random structural changes».

During the last years a better explanation has been obtained of the ROBERTSON law, since it has been found that the centromeres are, in fact, capable of splitting in two. However, this is not so simple as the oldest conception of this phenomenon made out. UPCOTT (1937 b) discovered that a centromere can at times split transversely, by so-called misdivision, in trivalents and univalents in triploid *Tulipa*. RHOADES (1938) was able to split a centromere in halves by means of X-rays in the same way, thus producing two chromosome fragments, each with a terminal centromere or, more correctly, a centromere half. Later investigations (DARLINGTON 1940 b, RHOADES 1940) have shown that such a half of a centromere is not quite able to function. However, by union of the two centromere halves of two daughter chromosomes (chroma-

tids), a chromosome can be formed which has two identical arms and a *whole* centromere. This type of chromosome is called an iso-chromosome.

A number of other investigators have also found that a breakage of the centromere may actually take place, where also iso-chromosome formations have been observed later in at least a few instances. (KOLLER 1938, HÅKANSSON 1940, PROPACH 1940, LEVAN 1942, and MÜNTZING 1944). Furthermore, KARPECHENKO (1940) arrived at similar results at colchicine treatment. It has not as yet been fully explained whether or not the so-called T-phenomenon (see, *e. g.*, PRAKKEN and MÜNTZING 1942) is due to the breakage of a centromere, causing fragment-centromeres to function also in an ordinary »monocentric» chromosome. Finally, GILES (1944) has found that iso-chromosomes can appear by crossing-over in peri-centric inversions.

The significance of the iso-chromosome in changes in the chromosome numbers, in accordance with the ROBERTSON law, is illustrated by the following case. Supposing that we have a normal two-armed chromosome with arms a and b. This chromosome can then, by means of a centromere breakage, form the telocentric chromosomes a and b. However, these chromosomes are not stable, but may by »fusion» of the centromere halves give rise to a pair of two-armed chromosomes, *viz.*, a—a and b—b. When such a chromosome is introduced into a genome, disturbances to the genetic balance should occur owing to the fact that the arms a and b are represented three times instead of twice. This disturbance should be severe and every loss-mutation in one of the arms should, therefore, be favoured by natural selection. Thus, we obtain quite soon two practically rod-shaped chromosomes with arms a and b, respectively. The chromosome number has in this way increased, but the number of chromosome arms remains unchanged.

That the iso-chromosomes can actually play this part is obvious from the fact that they are frequent in *Datura*. This has long been known. In addition, certain signs indicate that the Y-chromosome of the *Drosophila* is really an old iso-chromosome (DARLINGTON 1940 b).

Accordingly, light has now, without any doubt, been shed on the mechanical background of the ROBERTSON law. There are two different possibilities of chromosome increase, *viz.*, translocations and the introduction of small, supernumerary, and inert chromosomes comprising practically only one centromere, on the one hand, and changes via the iso-chromosomes, on the other.

Summing up, it may be said that we know the mechanism of the morphological chromosome changes and of the variations in the chromosome numbers. It has also been established that these two changes are counteracted by a more or less effective sterility barrier in the heterozygotes. Consequently, it is quite natural that, in a series of species, the chromosome morphology is

in several instances similar, in spite of the marked genetic difference between the species. When morphological differences occur between closely related species, it follows that also these changes should be comparatively slight.

After the above recapitulation of our present knowledge of the constancy of the chromosome morphology, I will now proceed to discuss the chromosome morphology of the Swedish Salmonoids.

Firstly, as regard the chromosome number, the Swedish species can be divided into three different groups. The first group consists of salmon and smelt with n = 30 and 29, respectively. The second group is the largest one, comprising brown trout, char, gwyniad and small gwyniad, all with the haploid chromosome number of 40, and speckled trout with the number 42. The third and last group contains the grayling with the number n = 51.

Furthermore, the close relationship between the various species is apparent from the similarity of the chromosome morphology. Thus, all the species have a minority of V-shaped chromosomes and a majority of rod-shaped ones. The frequency of V's and rods is evident from the following survey.

	Group 1	Group 2	Group 3
Haploid chromosome number	29 or 30	40 or 42	51
V-chromosomes, number of	5 or 6	8	14 (uncertain)
Rod-chromosomes, number of	24	32 or 34	37 »
Number of species	2	5	1

As already mentioned, the frequency of V's and rods in the *Thymallus* is uncertain, owing to the shortness of the chromosomes and their metaphasic positions. However, the following definite and useful conclusions may be drawn from this survey:

1. The variation of the chromosome number *within* the three groups is insignificant.

2. The difference between the three groups is approximately 10 chromosomes.

3. The chromosome number in each group is almost a multiple of 10, *i. e.* 30—40—50.

4. As the chromosome number increases, V-shaped chromosomes will become more numerous — contrary to the Robertson law.

Evidently, the chromosome numbers and the chromosome morphology of the Swedish Salmonoids disclose two contrasting tendencies, viz., a marked constancy, on the one hand, and violent sudden variations, on the other.

The chromosome constancy is principally illustrated by the fact that the

variations within the three groups of species are but slight, and that, for instance, the chromosome number of gwyniad, small gwyniad, brown trout and char is not only the same but that this also applies in almost every detail to the morphology, being 8 V-shaped chromosomes and 32 rod-shaped ones. Further illustration of this constancy is obtained from the cases reported earlier, where populations, which had been isolated from one another for eight or ten thousand years, reveal, this notwithstanding, no ascertainable chromosome differences (with the exception of salmon).

The opposite tendency, *i. e.* violent chromosome variation, is perhaps best exemplified by the species pair salmon and brown trout, which are systematically very closely related (it is very difficult to distinguish between them in their early stages). This taxonomic conception finds support in cytological data, the chromosomes pairing well in the F1-hybrid which also gives fertilizable milt, as well as grains of roe, also fertilizable, although F2 probably always dies owing to mitotic disturbances. However, these closely related species show the haploid chromosome numbers 30 and 40, respectively, or, if the chromosome arms are counted instead, 36 and 48, respectively.

The ascertained chromosome constancy is well in agreement with the survey of mechanically conceivable possibilities of chromosome variations.

The marked group differences, *i. e.* the 10-chromosome-leap, contrast equally convincingly with the possibilities of chromosome variations discussed earlier. Thus, differences as large as those between smelt and grayling are exceedingly rare in one and the same family in the animal kingdom. In view of the difficulties of stabilization of new chromosome numbers it can be safely maintained that a change of 10 or 20 chromosomes in the haploid number *cannot have taken place step by step but rather by leaps, i. e. through polyploidization. This is confirmed, above all, by the fact that the variation cannot have occurred in accordance with the Robertson law, since the number of V-shaped chromosomes also increases in proportion to an assumed multiple of 10 chromosomes.*

It has earlier been pointed out (p. 98) that an internal genetic balance is still noticeable in separate nuclei in one and the same embryo, causing the nuclei with a chromosome number of approximately 20, or multiples thereof, to have the best mitotic activity. The basic number (x) of the Salmonidae family cannot, however, be 20, owing to the fact that the salmon would then be a triploid and the grayling a pentaploid, which is quite inconceiveable. The basic number must instead be 10 and the Salmonoids, accordingly, high-polyploids, according to the following survey.

Osmerus eperlanus ..	6x — 2	= 58
Salmo salar	6x	= 60
Salmo trutta	8x	= 80
Salmo alpinus	8x	= 80
Salmo fontinalis ...	8x + 4	= 84
Coregonus lavaretus	8x	= 80
Coregonus albula ..	8x	= 80
Thymallus thymallus	10x + 2	= 102

The above survey shows how the two contrasting tendencies, *viz.*, chromosome constancy and violent chromosome differences, can easily be accounted for by polyploidization and normal »fragmentation».

It remains to be established whether or not there is still any Salmonoid with a chromosome number of 20 or 40. Apart from the species examined by the present author, the following investigations also refer to this problem.

MAKINO (1937) subjected to analysis the species *Oncorrhynchys keta Walb,* which is closely related to the salmon. He found that the diploid chromosome number equalled 74. Although this is, undoubtedly, of the correct order of magnitude, I am not convinced that the number is quite exact. His examination concerned primordial germ cells, the nuclei of which are big but much smaller than the embryonal nuclei. Accordingly, there are, no doubt, great difficulties entailed in the ascertainment of such a high chromosome number.

POMINI (1939) studied several Italian forms of brown trout. In view of the present lack of certainty with regard to the taxonomy of these species, it has not, in my opinion, been definitely proved that any of these species differ from that of *Salmo trutta.* POMINI'S chromosome numbers were, moreover, uncertain, as he himself remarks. Still, the order of magnitude is correct.

The older data concerning the chromosome number of the rainbow trout (see p. 11) must be regarded as unsatisfactory. The material of this kind at my disposal has been very restricted and I am, therefore, as yet unprepared to state an exact chromosome number in this case. Nevertheless, this much may be said, that the diploid number far exceeds 24, which was reported by the older authors.

The Salmonoid fish family has a number of species in North America. It will be interesting to determine their chromosome numbers. Judging from the *Salmo fontinalis,* which is originally an American species, the chromosome numbers must be high, and the likelihood of coming across such a species with original numbers may, perhaps, be small.

According to BERG (1935), the first Salmonoids appeared in the *Miocene*. The family is, apparently, very old and the repeated polyploidizations which must have occurred in the phylogeny of the family probably date far back.

B. Meiotic disturbances.

Polyploid forms are often subjected to meiotic disturbances owing to multivalent formation. This refers especially to all auto-polyploids and to allopolyploids as well when the parental species do not differ too greatly from one another with regard to their chromosomes.

In spite of this, auto-polyploidy is an important evolutionary factor in the vegetable kingdom (MÜNTZING 1936). In addition, it has been found that the frequency of multivalents is sometimes not particularly great (MAKINO 1939 b, MÜNTZING and PRAKKEN 1940), and that a decreased multivalent formation and with it increased fertility can be obtained by means of recombination between different auto-polyploid families (MÜNTZING 1943). The contrasts between the experimental polyploids and the spontaneous ones in ordinary, natural surroundings have been modified, especially after WETTSTEIN'S and STRAUB'S (1942) fine *Bryum* study, where an auto-polyploid at first showed giant growth and high sterility, but lost its giant form after several generations and regained full fertility. No doubts can be entertained regarding the fact that natural selection reduces the cellular size and increases the frequency of bivalents.

Irrespective of whether the Salmonoids are auto- or allopolyploids, we can hardly expect their polyploid origin to manifest itself by multivalent formation.

As mentioned earlier with regard to the various species, some information has been obtained, in spite of the considerable technical difficulties, of the meiosis of the Salmonoids. Several species were found to be characterized by a comparatively high percentage of disturbed meiosis metaphases, where principially the univalents were observable. The important question of whether the occurrence of univalents coincided with that of the multivalents (trivalents) could, unfortunately, not be given a straight answer. However, in a few cases the occurence of trivalents was either probable or certain.

The occurrence of univalents may be due to a structural hybridity. For reasons set forth above, this is less likely in the case of the Salmonoids.

Furthermore, the occurrence of univalents may depend on a low chiasma frequency. At in-breeding, a reduced chiasma frequency was noted by RANDOLPH (1928), MC CLINTOCK (1929), BEADLE (1933), DARLINGTON

(1934), LAMM (1936), and PRAKKEN and MÜNTZING (1942). Probably this applies also to the low chiasma frequency in the *Ascaris* (JEFFREY and HAERTL 1938). Nor can it be excluded with regard to the Salmonoids. On account of the fishes' habit of returning to the same place for spawning and owing to the considerable chance losses during the ontogeny, inbreeding can, conceivably, easily occur in small populations in running water.

In addition, univalents and multivalents may appear as the result of numerous more extensive duplications within the chromosome set. (Related problems have recently been discussed in detail by LEVAN 1942, 1945.) The conclusion may be drawn, from LEVAN'S investigations, that duplications are not so frequent as assumed earlier. As a rule, such duplications permit pairing with chiasma formation only when the pairing competition has been reduced, *e. g.*, in haploids and in hybrids.

Should future research be able to confirm the occurrence of trivalents and other multivalents in the normal meiosis of the Salmonoids, it would imply that the univalents are not dependent on a low chiasma frequency, but appear owing to the multivalent formation. In this way, further indication would be obtained of the polyploid origin of the fishes. At present, this indication is rather weak. We have to be satisfied with merely stating that there is nothing in the meiosis of the Salmonoids contradicting an assumption of their polyploidy.

C. The significance of systematical variation.

»Was die Habichtskräuter, die Rosen und die Bromberen in der Botanik, das bedeuten die Gattungen *Unio, Anodonta, Coregonus* und *Salmo* in der Zoologie. Ihre Systematik ist so verworren, dass es bisher nicht gelungen ist, jene Ordnung herzustellen, die den Systematiker befriedigt» (STEINMANN 1941, p. 525).

This statement — which all taxonomists will undoubtedly endorse — will immediately show that some of the Salmonoids are in a class of their own as regards taxonomic difficulties. Several factors play a part in this connection, *viz.*, the occurrence to a large extent of modifications, complicated by the fact that sexual maturity may set in at widely different times and sever further development in a morphological respect. In salmon, the sexual maturity is manifested at such an early phase as to justify the term paedogenesis (SHAW 1840, and many later authors. For further details see ALM'S work, 1943). Apart from these complications, also another variation occurs which cannot be entirely explained by modifications, or the like. In illustration of this, it may be mentioned that not less than five different

forms of the *Coregonus* occur in one single lake in Lapland in the very north of Sweden, with different appearances, different manners of growth, different numbers of gill rakers and scales, different spawning times and places. These different forms must have become differentiated in a comparatively short time, probably after their isolation in the lake less than ten thousand years ago.

Considerable attention has been devoted to the task of explaining the variety of forms of the Salmonoids. Some ichtyologists have imagined that the Salmonidae family is at an unusually lively phase of its evolution. It does not seem unlikely that periods of slow evolution can alternate with periods of acceleration as, for instance, when a group of animals are introduced into a new geographical area where its selective value is quite different (cf. HUXLEY 1942, who discusses such cases in detail).

However, there are also other conceivable explanations of this phenomenon of accelerated evolution, *i. e.* polyploidization. The original purpose of the present work was, as already stated, to examine whether or not a cytological explanation could be obtained of the great systematical variation of the Salmonoids. It is, therefore, interesting to find that, *e. g.*, the gwyniad, which has the most marked variation among all the species, also discloses the greatest cytological »disturbances». The regular frequency of embryos with deviating chromosome numbers and abnormal embryonic development may either be due to an unusual multivalent formation in the meiosis and, consequently, a high frequency of gametes with an unbalanced chromosome number, or else be explained by a high frequency of spontaneous chromosome re-arrangements. Which of these explanations is the correct cannot at present be determined. However, it is evident that the marked variation is connected with a lively re-arrangement of the gene material. The *morphological variation of the gwyniad has in this way obtained a plausible cytological explanation.*

The same explanation may apply also to other species with a smaller variation. Still, it is necessary that the genome is not too well balanced in order that a lively re-arrangement shall give rise to a high frequency of gametes and zygotes fit for life. Nowadays, it is well known that a polyploid organism is much more tolerant as regards chromosome re-arrangements than a diploid organism. This is, of course, due to the fact that the polyploid organism is less liable to lethal genetic lack of balance, on account of its having more than two of all kinds of genes. For the same reason, recessive gene mutations are less easily manifested in polyploids (HUSKINS 1941). Several of the taxonomic characteristics of the fishes, or perhaps all of them, are regulated by the polygenes (cf. SVÄRDSON 1944). In this way,

new mutations may more easily affect the phenotype. According to KOSTOFF (1939 b), the greater tendency of the polyploids towards variability may also involve modifications.

In the opinion of the present author, the marked systematic variation of the Salmonoids is therefore due to a lively re-arrangement of the chromosomes. This derives evolutionary significance from the fact that the animals have, in the capacity of old polyploids, a considerable power of endurance against a genetic lack of balance.

D. Polyploidy and sex.

It was MULLER (1925) who, on the basis of BRIDGES' discovery of the mechanism of sex determination in the *Drosophila,* laid down the rule that polyploidy in animals must be extremely rare and, in actual fact, only occur in not bisexual animals, or animals with another mechanism of sex determination. Rarely has a scientist in a prophecy of the future — as at that time it was — won such unanimous support from his colleagues. In all the modern comprehensive surveys, such as DARLINGTON (1937), DOBZHANSKY (1937), STURTEVANT and BEADLE (1939), HUXLEY (1942), and BAUER and TIMOFEEFF-RESSOVSKY (1943), the absence of polyploidy in the animal kingdom is still regarded as an absolutely fixed rule, confirmed merely by the small number of exceptions formed by the polyploid parthenogenetic organisms.

However, MULLER has now somewhat revised his rule, or »law», and it is quoted below in extenso in its new form (MULLER 1941, p. 245):

»The hindrance to establishment of polyploidy ... lie chiefly in (1) the fact that in triploids a relative dosage of sex-determining genes like that in the heterozygous sex (*i. e.* a dosage of sex-chromosomal to autosomal genes of 1:2 cannot exist; (2) that in triploids, the irregularities of segregation lead to few normal progeny; and (3) that in tetraploids of heterozygous sex, were they to appear, the two like sex-chromosomes of major value (X or Z) would tend to segregate from one another, with the resultant production of few or no gametes having normal ratio of X (or Z) chromosomes to autosomes.

In some groups, however, special conditions might exist which reduces the seriousness of the above difficulties. For instance, there might be a greater range of dosage relations compatible with the production of fertile individuals of the heterozygous sex, or the direct step to tetraploidy, without triploids as an intermediate stage, might occur oftener, and some special mechanism, such as the presence of a single Y-chromosome of double segregational

strength, might send the two like sex-chromosomes of major value to the same pole. Such a group, perhaps, is that of the *Hemiptera heteroptera,* in which SLACK (unpublished) has obtained evidence indicating that polyploidy has occurred independently a number of times.»

Here MULLER bases his contention on the mechanism of sex determination in the *Drosophila.* According to this interpretation, the Y-chromosome lacks, as is known, sex-determining genes, although genes do occur which are connected with the fertility of the male. MULLER'S first point is a direct generalization of the results of the *Drosophila* investigations so as to render them applicable also to other organisms. He admits that other organisms may not be equally sensitive to this balance. His point number three, *viz.,* that the tetraploid XXYY should produce XY-gametes to a predominating degree, has been experimentally tested and verified by WESTERGAARD (1940), among others. These gametes from the heterozygous sex should, therefore, produce zygotes of the types XXXY and XXXX, after fertilization with the gametes from the homozygous sex. MULLER was not able to predict that these two combinations would represent the two sexes, since this would presuppose the occurrence of strong sex genes in Y, which does not conform with the mechanism of the *Drosophila.*

MULLER'S second point is of indisputable validity. His own softening of the statement that the step towards tetraploidy may be direct is, accordingly, necessary with regard to the possible occurrence of tetraploid, bisexual animals. The great difficulty is, of course, the fact that such a chromosome multiplication must take place simultaneously in several animals, since one single tetraploid cannot — as in the case of plants — vegetatively give rise to a clone. This constitutes, perhaps, the main difficulty of stabilization of polyploid, bisexual animals.

The generalization of the mechanism of the sex determination of the *Drosophila* has been found incorrect. A number of polyploid, dioecous plants occur in the vegetable kingdom. They seem to speak against MULLER'S rule. However, in the majority of cases, it has not been possible to decide whether their dioecism has occurred *after* they became polyploid, or not. Definite cases have been ascertained in the Melandrium (ONO 1939, 1940 a, 1940 b, WESTERGAARD 1940, WARMKE and BLAKESLEE 1940 a, 1940 b, 1941). It has been clearly established that the organism XXXY in *Melandrium* is male, and that dioecism is possible in the tetraploid form. After these experimental observations, LÖVE (1942) came across tetraploid, dioecious *Melandrium* in ordinary, natural conditions.

The same mechanism as in the *Melandrium* has recently been noted in the *Rumex subgenus Acetosella* (LÖVE 1944). In this sub-genus, a number of

polyploids occur and the X-chromosomes appear at present to be in a state of gradual transition to autosomes. A marked epistatic male factor exists in the Y-chromosome. This mechanism of sex determination also resembles that of the Amaranthaceae (MURRAY 1940), representing no hindrance to polyploidization. The modern investigations of the mechanism of sex determination in plants have been compiled and subjected to detailed analysis by ALLEN (1940), KUHN (1942), and KNAPP (1943). These recent studies show, with comparative emphasis, that MULLER'S rule does not refer to plants, at any rate not to the extent of rendering dioecism an absolute hindrance to polyploidy.

As to the question of the polyploidy of the Salmonoids, the mechanism of sex determination is, as a matter of course, of fundamental importance. Unfortunately, we are as yet quite ignorant on this point, although a number of investigations have been carried out on other fishes, and, principally, on aquarium fishes.

It is not easy to state the actual position with regard to this problem, since no agreement is to be found in the matter at the moment, opinions differing strongly between the representatives of the »classical school», on the one side, and the whole number of those who have devoted particular interest to the mechanism of sex determination of fishes, on the other.

WINGE (1922, 1930, 1932, 1934, 1937) studied *Lebistes reticulatus* and arrived at the conclusion that the male is heterogametic. Further, he contended that the Y-chromosome has a marked male sex factor, but that all the autosomes also contain sex genes, some of which act in a male direction, and others in a female direction. He succeeded, in fact, in producing XY-females and YY males, as well as XX males. In the latter case, the whole population had XX and the sex determination had been taken over by an earlier autosome pair.

AIDA (1936) obtained similar results on *Aplocheilus*. He presented, on the basis of these results, a new theory of sex determination. However, GOLDSCHMIDT (1937) considers this theory to be »essentially the same as the one which we derived from the *Lymantria* formulations, though couched in different language» (p. 438).

The most comprehensive work in this field of research was performed by KOSSWIG (KOSSWIG 1931, 1932, 1933 a, 1933 b, 1933 c, 1934, 1935 a, 1935 b, 1935 c, 1936 a, 1936 b, 1936 c, 1937 a, 1937 b, 1939 a, 1939 b, 1941), BREIDER and KOSSWIG (1937), and his followers BREIDER (1935 a, 1935 b, 1936 a, 1936 b, 1937, 1939, 1942), SCHWIER (1939) and RUST (1939, 1941). Also American investigators have taken part (BELLAMY 1936 and GORDON 1937). It is not an easy matter to give a brief report of KOSSWIG'S con-

ception of the mechanism of sex determination. Accordingly, RUST's (1941) interpretation of KOSSWIG's theory, being the most lucid one, will be followed here.

Species occur which are either polyfactorial or monofactorial with regard to sex determination, the former being the most primitive. Sex is determined by the interplay of autosomal genes, *i. e.* so-called T-genes, which act as polymeric factors. The T-genes are »Anlagen, die die Empfänglichkeit eines potentiell bisexuellen Organismus für vermännliche oder verweibliche Aussenbedingungen beeinflussen» (RUST 1941, p. 338). No sex-chromosomes (gonosomes) whatsoever are ascertainable in these species. Apart from the T-genes, the environment may influence the sex ratio. The total sum of the T-genes determines the sex. For instance, all combinations between T^0—T^1 can be defined as weak or female, and those between T^1—T^2 as strong and male. Moreover, the effect of the T-genes may sometimes pass over T^2 to T^3. The strength of the T-genes will then no longer be unstable with regard to the environment, but will directly determine the sex.

Individuals with T^3-genes constitute the *homogametic* sex of the »monofactorial» species. These T-genes are counteracted by a sex-determining gene in one chromosome which — in a simple dosage — compensates for the T-genes, thus presenting, apparently, a simplified picture of back-crossing according to the monohybrid scheme. The monofactorial species, consequently, derive directly from the polyfactorial ones, which are latent hermaphrodites with a varying sex ratio.

Evidently, KOSSWIG's conception covers altogether the results arrived at by WINGE and AIDA. Moreover, WINGE's opinion is much alike.

WINGE (1937) attempted, on the basis of his view of the mechanism of sex determination, to explain GOLDSCHMIDT's findings in investigations on the *Lymantria*.

GOLDSCHMIDT (1937), on his part, violently attacks KOSSWIG's conception, that »such things exist as phenotypic sex determination, male and female heterozygosis within the same genus, empty X-chromosomes, female determiners in the Y, multiple autosomal male determiners, etc.» (GOLDSCHMIDT 1937, p. 434). He also submitted WINGE's and AIDA's results to criticism.

Another attempt to explain KOSSWIG's results, in accordance with the generally accepted mechanism of sex determination, *i. e.* that of the *Drosophila* and the *Lymantria*, was made by HÄMMERLING (1937 a, 1937 b) whose results differed in an interesting way from those of GOLDSCHMIDT.

GOLDSCHMIDT's results, as well as HÄMMERLING's, have been exposed to overwhelming criticism by KOSSWIG (1939 a, 1939 b, 1941), RUST (1939,

1941), SCHWIER (1939) and BREIDER (1942). From an outsider's point of view, this controversy clearly shows that GOLDSCHMIDT has not been able to explain all results of the KOSSWIG school, nor has HÄMMERLING.

Arguments particularly favouring KOSSWIG'S theory are to be found in the indisputable results permitting the production, by selection, of families with a low and a high percentage of males, respectively, in the *Xiphophorus* and the *Limia* species. Furthermore, KOSSWIG has found that the sex genes are probably pleiotropic and also affect the colours and the speed of sex differentiation, causing the appearance of »dwarf males» and »giant males». This is in fair agreement with the complicated question of the time of sexual maturity in fishes (cf. SVÄRDSON 1943).

The problem of intersexuality in fishes also provides difficulties. In all likelihood, all the Teleosts go through a protogynous hermaphroditic stage (SCHWIER 1939, SVÄRDSON and WICKBOM 1942). Sex transformation in grown animals is not infrequent and may, probably, often be interpreted as a delayed juvenile, transitory intersexuality. The literature on this subject is too extensive to be dealt with in this connection. However, it may be stated summarily that *permanent intersexuality, owing to a genetic lack of balance is rare while, on the other hand, a good deal speaks in favour of the fact that a fertile individual of one sex may transform to a more or less fertile individual of the other sex.* The balance between the sexes in the mechanism of sex determination decides itself one way or the other, so to say, on »the razor's edge» (cf. KNAPP 1943).

The recent investigations of sex determination regarding the *Betta splendens* (EBERHARDT 1943) offer good evidence of the labile state of the sex in fishes. The *Betta* has a normal sex ratio, *i. e.* 1:1, only at optimal environmental conditions. Unfavourable environment, *i. e.* concerning food, space, and water conditions, favours differentiation in a male direction, causing a statistically certain predominance of males. EBERHARDT was able to show that, by determining the mortality rate under such unfavourable conditions, the male surplus does not take place by selective mortality among the females. The influence of environment versus genetic sex determination may set in as late as at an age of 4—6 weeks.

As regards the Salmonoids, it has so far only been established that the *Salmo trutta* and *fontinalis* have a sex ratio of 1:1 (SVÄRDSON unpublished). Information has been given of an abnormal sex ratio out in the open of a thousand males to one female (NERESHEIMER 1937). Since investigations have already been performed with regard to several different fish families *(Cyprinodontidae, Anabantidae)* and widely separated species and, further-

more, since these investigations (on aquarium fishes) show results emphatically in favour of KOSSWIG'S conception, it seems probable, in the opinion of the present author, that the same mechanism of sex determination should, for the present, be assumed also concerning the *Salmonidae* family.

Thus, it appears evident that the mechanism of sex chromosomes in fishes — in cases where it can be said to exist — has a strong sex gene in the Y-chromosome, similar to conditions in the bisexual plants. Furthermore, considering the fact that, according to KOSSWIG, the X-chromosomes lack normal sex-determining genes, apart from their supply of T-genes, there is, apparently, no hindrance for the XXXY fishes being males and the combination XXXX giving females — as in the case of the *Melandrium*. MULLER'S *point no. 3 is, consequently, invalid as a hindrance to the stabilization of polyploidy in fishes.*

Nor should the internal balance of the sex genes in relation to the autosomal genes in a triploid preclude the possibility — after what has already been inferred — that triploid fishes can be males as well as females. MULLER'S *point no. 1, consequently, lacks validity as a hindrance to polyploidization in fishes.*

MULLER'S point no. 2, *viz.*, irregularities of segregation of triploids as an intermediate stage in polyploidization — is, on the other hand, indisputable. Tetraploids must have appeared with, at the most, one generation of triploids as an intermediate step. The occurrence of polyploids in the Salmonoids may, conceivably, have taken place according to the following hypothetical outline.

A Pro-Salmo population with 20 chromosomes (diploid) has been split into two populations, owing to climatological or geographical reasons. The populations have been isolated from one another for a few ten thousands of years, during which time some structural changes within respective chromosome-sets have become homozygotic. After new changes in the geographical or climatological conditions (glacial periods!), the populations have again met along a delimitation line. On this line, crossings in the form of hybrid swarms (cf. HUXLEY 1942) have occurred. The heterozygotes have revealed an abnormally high frequency of unreduced gametes on account of meiotic disturbances. The eggs among them have been capable of producing a fairly high frequency of triploids. By means of random fertilization, of eggs with varying numbers of T-genes, the triploids have become bisexual. Triploid females have presented a high frequency of triploid eggs — again owing to meiotic disturbances — which have produced tetraploid Pro-Salmo after fertilization. Random T-gene combinations have again permitted the tetraploids to become bisexual. The fertility has been reduced at first, but a

positive selection factor (endurance to cold?) has counteracted this low degree of fertility. The fertility has gradually improved and the tetraploids have, accordingly, begun to oust the diploid original forms.

This process must have taken place several times, and the boundary between auto- and allopolyploidy in this case breaks down. The hexaploids and the decaploids have been formed in a similar way. Polyploidization has been greatly favoured by natural selection which partly caused an unusually high polyploidization, and partly altogether exterminated the originally diploid species, *i. e.* those with 20 chromosomes.

In this way, MULLER'S point no. 3 has either been put out of play or made altogether invalid. Thus, summing up, it may be said that MULLER'S *famous law does not place insurmountable obstacles in the way of the polyploidization of fishes.*

E. Polyploidy in the animal kingdom.

Polyploid forms in the animal kingdom have rarely been reported. This seems to confirm MULLER'S rule. The known cases are, apparently, as follows:

Artemia salina, tetraploid and octoploid species (ARTOM 1925, BARIGOZZI 1934, 1940, GROSS 1932).

Trichoniscus provisorius, triploid parthenogenetic species (VANDEL 1926, 1927, 1940).

Solenobia pineti and S. triquetrella, triploid and tetraploid parthenogenetic species (SEILER 1927, SEILER and SCHÄFFER 1941, SEILER 1942).

Curculionidae, 8 species polyploids, 5 triploids, 3 tetraploids, all being parthenogenetic (SUOMALAINEN 1940 a and 1940 b).

These cases are the most widely and best known ones. All the polyploid forms are parthenogenetic, and it is either probable or certain that the parthenogenesis has occurred first in all of them, the polyploidy setting in later. This is mainly borne out by the fact that also diploid parthenogenetic forms exist among similar or closely related species. SEILER (1942) has in the *Solenobia* studied the meiosis and the first cleavages. He found that the anaphase of the second meiotic division has either failed, or the polar body has »fertilized» the nucleus of the egg. The tendency towards fusion between the cleavage nuclei very frequently leads to polyploidy. The *Daphnia* have sometimes been considered to be polyploid, which is, however, repudiated by MORTIMER (1936).

In accordance with MULLER'S rule, not only parthenogenetic animals, but also hermaphroditic ones should disclose greater possibilities of polyploidization within the phylogeny. WHITE (1940 a) has, therefore, made a compilation of probable or possible cases of polyploidy within these animal groups. He found that polyploidy evidently does occur, though but rarely. Among the *Rhabdocoela,* there are 17 possible polyploids out of 65 examined cases (cf. RUEBUSH 1938), among the *Pulmonata* possibly one out of 29 species (*i. e.* the *Helix pomatia*). The *Hirudinea* and the *Oligochaeta* reveal possibly 2 in either group. PEACOCK has also described a probable case in a Mollusc species (PEACOCK 1940).

Finally, LORKOVIC (1941) presented indisputable evidence of the occurrence of polyploidy among butterflies in the genera *Polyommatus, Leptidea* and *Erebia,* where separate species have chromosome numbers which are multiples of each others. It should be noted that these animals are, nevertheless, bisexual and LORKOVIC'S investigation is, therefore, of considerable value. He rejects earlier interpretations of fragmentation and much attention has been attracted to his results.

MAKINO (1939 a) found 104 chromosomes in the carp, while its closest wild relatives had only 52. However, he does not seem to draw any conclusions at all from this finding as regards possible polyploidy.

VANDEL (1937) has listed a great number of animal species where the chromosome numbers constitute more or less exact multiples of each other. His survey is of interest and should be referred to for details. VANDEL writes, as follows: »I have been struck by the fact that adjacent species, or species belonging to the same systematic group, frequently show chromosome numbers in which one is twice the other ... These examples fall into two very distinct categories, some showing polyploidy, others, more numerous, showing fragmentation» (VANDEL 1937, p. 519). He continues, further on: »What seems least to have engaged the attention of biologists is that frequently the phenomenon of fragmentation is concerned with the *whole of the chromosome stock,* involving, by this fact, a doubling of the chromosome number. The conditions which result from the simultaneous fragmentation of all the chromosomes bear a singular resemblance to those which are the result of polyploidy, and only careful study allows one to distinguish the two methods.

The two methods can be distinguished by the following characters: —

(1) In polyploidy the chromosomes of the diploid form and those of the polyploid form maintain the same size and shape. But the size of the

nuclei of the polyploid forms is greater than that of the diploid form (BOVERI'S law).

(2) In the case of fragmentation, the chromosomes have a different form from that of the type form, and their size is less. But the size of the nuclei is similar in the two cases, the total quantity of chromatin remaining the same.»

VANDEL'S work has been quoted at some length owing to the fact that his results regarding polyploidy in the animal kingdom are included in several literature lists, and in view of the great importance attributed to his principal result, *viz.*, »polyploidy is rare in the animal kingdom» (p. 535). However, it is surprising that he has not been subjected to more severe criticism for his hypothesis of simultaneous fragmentation of the whole chromosome-set.

The two »methods» by means of which — according to VANDEL — a distinction can be made as between polyploidy and simultaneous fragmentation are of very weak evidential value. We know nowadays (see, for instance, WETTSTEIN and STRAUB 1942) that the nuclear size in an old polyploid may be forced down to approximately the volume of a diploid by natural selection. Moreover, the size of the nucleus and that of the chromosomes in the Salmonoids has been found capable of considerable variations. As regards the form of the chromosomes, VANDEL'S theory may, of course, be correct, but since the majority of chromosome determinations are performed in investigations of the meiosis — where the chromosomes show but little of their morphology owing to the marked spiralization — this argument is not, in most instances, of very much value. Accordingly, the usefulness of VANDEL'S two »methods» will be left open to discussion. As mentioned earlier, at present, *knowledge is lacking with regard to a mechanism which would be capable of producing simultaneous fragmentation of a whole chromosome-set.* VANDEL'S construction that multiples of chromosome numbers might be explained by means of two different mechanisms, *viz.*, polyploidy and fragmentation, thereby falls to pieces.

Polyploidy is the most probable and, in the majority of cases, the only possible explanation. VANDEL'S list of chromosome multiples should, therefore, be regarded as a list of the polyploid cases hitherto observed in the animal kingdom, in which case they would not become so infrequent.

In this connection, I should like to stress another point, in particular. In order to discover polyploid animals, the groups where different species occur with greatly varying chromosome numbers have been studied. However, since the majority of the lower animal groups are of an exceedingly high age,

it appears, in the opinion of the present author, very likely that, in several instances, *a whole systematic group will have approximately the same chromosome number and still be polyploid, while the phylogenetically older types with lower chromosome multiples are altogether extinct. Old polyploidy, from a phylogenetic point of view, is practically impossible to discover in recent animals.*

The basic number of the Salmonoids (x) is 10, the number of chromosome arms equals 12. This basic number will be found within widely different vertebrate groups. However, VANDEL (*op. cit.*), who lays particular stress on 12 as »the basic number», considers it probable that the fragmentation has caused »a very fine example of increase in chromosome number going along with the evolution of the group» (p. 535). On the other hand, personally, I should not be surprised if polyploidy would one day be considered equally important in animal evolution as it is now in the vegetable kingdom.

VI. Summary.

1. The examinations of the chromosomes of the Salmonoids have been performed with the help of a smearing technique. Dissected embryos, or testicles, have been fixed in acetocarmine, being in this way preliminary stained. The smears have been carried out on a slide coated with a film of albumin glycerine. After the removal of the cover-glass in alcohol, staining has taken place in a thermostat in acetocarmine. Then, the preparation has been rendered permanent by treatment with Canada balsam. These preparations are very durable.

2. Good mitotic pictures of metaphases showed that the chromosomes underwent a considerable variation in size. This variation has been subjected to statistical analysis. In successive metaphases, the chromosomes become shorter and narrower, while the change in form within a mitosis (prophase-metaphase), as is known, consists of shortening and thickening. These two variations are not proportional, in contrast with the general conception. Instead, a long chromosome arm regularly becomes proportionately shorter than a short arm. Thus, an arm index is not an absolute criterion of the morphology of a chromosome. The reason for the variations in shape must be spiralization. It has been suggested that a shorter chromosome arm can more rapidly attain a certain degree of spiralization. During the final stages of the spiralization, therefore, the long chromosome arm will become shorter, according as it catches up with the spiralization start of the shorter arm.

The breadth of a mitotic chromosome arm is not a function of its degree of spiralization but is, probably, in the first place, dependent on the amount of nucleic acid included in the chromosome.

3. The centromeres are invisible. Their division at the onset of the anaphase is asynchronous. Consequently, a moderate lagging of some of the chromosomes is normal in mitotic anaphase.

4. Secondary constrictions are frequent. They are not constant in number, nor in position, but vary considerably. In this way, definite homologization of the homologous chromosomes is either rendered more complicated, or impossible. The number of constrictions is highest in the prophase, then falls pronouncedly and, at anaphase, there are, as a rule, none left. Constrictions

are interpreted as parts deficient in nucleic acid (probably heterochromatic), with retarded nucleic acid metabolism and also retarded spiralization.

5. Non-disjunction is common in the mitotic anaphase, particularly in the *Coregonus lavaretus*. The primary reason for non-disjunction seems to be reduced power of repulsion in the centromere halves which do not wander away from one another. Such a centromere also divides after the others. The somatic chromosome numbers shows, as a result of the non-disjunction, a variation around the diploid number.

6. In the metaphase plate (mitosis) — particularly distinct in the *Salmo alpinus* — a special orientation is observed, where the chromosome ends, the proximal as well as the distal ones, have such a conspicuous affinity to one another — possibly, deficient repulsion — as to cause conglomerates of chromosomes. Frequently, two rod-shaped chromosomes are placed in the form of a V. This phenomenon is probably caused by the heterochromatic parts.

7. The chromosomes of *Salmo salar* have a diploid number of 60, 6 pairs of which are V-shaped. Constrictions are numerous. The salmon populations on the west-coast of Sweden and in the lake Vänern (landlocked form) have the same chromosome morphology, which deviates on one point from that of the population in the Baltic. In the meiosis, 30 bivalents occur, although uni- as well as multivalents have been seen.

8. The *Salmo trutta* has a diploid number of 80 chromosomes, 8 pairs of which are V-shaped. Several different forms (by many authors referred to as subspecies, or even species) have the same chromosome morphology. The *Salmo trutta* has extremely few constrictions. In M 1, 40 bivalents occur, but uni- as well as multivalents are noted in 20—30 per cent of the cases.

9. The hybrid between salmon and brown trout has a diploid number of 70 chromosomes. The size of the chromosomes is an intermediary size between that of salmon (large chromosomes) and brown trout (smaller chromosomes). In the meiosis, serious disturbances occur with numerous univalents. The greatest number of observed bivalents is 30. The distribution of the chromosomes at A II cannot be studied but the chromosome numbers of the sperms can be determined at back-crossing to Salmo trutta. A variation has been established of 32—38 chromosomes, 35 being the most common number. Consequently, the univalents only perform one division and are, as a rule, not eliminated but included in the daughter nuclei at the meiosis. The back-cross hybrids disclose characteristic mitosis disturbances due to a lack of genetic balance.

10. These mitotic disturbances may be classified in the following categories. Multipolar spindles give varying chromosome numbers in the daughter nuclei. A series of different chromosome numbers present nuclei capable of producing mitosis. Anaphase sticking is very frequent. Chromosome elimination is met with and is, according to its nature, closely related with non-disjunction. Chromosomes and spindle show deficient co-orientation in space, as well as in time. The chromosome breaks increase, as is seen from fragments and new chromosome types.

11. The char, *Salmo alpinus* has a diploid number of 80 chromosomes, 8 pairs of which are V-shaped. Char-populations which have been isolated from one another for 8 000 years do not reveal any morphological differences within the respective chromosome sets. In the meiosis, disturbances occur as in other *Salmo* species.

12. The hybrid *Salmo trutta* × *S. alpinus* has marked meiotic disturbances. The number of univalents in M 1 equals approximately 20—30. M 1 degenerates and the hybrid is quite sterile.

13. The speckled trout, *Salmo fontinalis* has a diploid number of 84 chromosomes, 8 pairs of which are V-shaped. The set is characterized, *inter alia,* by a chromosome pair with an extremely long secondary constriction.

14. The hybrid *Salmo fontinalis* × *Salmo trutta* has still more conspicuous meiotic disturbances than the preceding hybrid. It is sterile and M 1 degenerates.

15. The gwyniad, *Coregonus lavaretus* has a diploid number of 80 chromosomes, 8 pairs of which are V-shaped. There are minor morphological differences between the various gwyniad forms. Marked non-disjunction, etc., renders morphological studies more difficult. In all the gwyniad forms, embryos occur which reveal mitotic disturbances, analogous to those of the back-cross hybrids (see above). Nuclei with different chromosome numbers are met with, mixed in all proportions with normal nuclei. Nuclei with approximately 20, 40 and 60 chromosomes seem, relatively speaking, the most common, and suggest an internal balance. About half of all the gwyniad embryos have a fragment chromosome. The fragment appears in all the examined forms. The most probable explanation is, apparently, that the fragment constitutes a sex-chromosome. In a survey of the literature, this is seen to be the first case of morphologically recognizable sex-chromosomes in fishes.

16. The small gwyniad, *Coregonus albula* has a diploid number of 80 chromosomes, 8 pairs of which are V-shaped. No definite fragment-chromosomes (sex-chromosomes) have been ascertainable, although fragments are

sometimes formed by breaking-up of the chromosomes. Ring-chromosomes also manifest themselves, as well as giant chromosomes, etc., all being the result of numerous spontaneous chromosome breaks.

17. The Grayling, *Thymallus thymallus* has a diploid number of 102 chromosomes, with probably 14 pairs of V-shaped ones.

18. The smelt *Osmerus eperlanus* has a diploid number of 58 chromosomes, 5 pairs of which are V-shaped.

19. Polyploid cells occur regularly in the somatic tissue of the embryos. Whether or not these cells may be assumed to have appeared owing to modified endomitosis has beeen discussed.

20. Spontaneous polyploid embryos are rare. Triploids of the *Coregonus* and the hybrid *Salmo salar* × *S. trutta* have been ascertained. Embryos, constituting mosaics of haploid, diploid and triploid nuclei have been found in *S. trutta*.

21. In order to elucidate certain mitotic disturbances, cold treatment has been performed. These experiments show that a low temperature may produce spindle paralyses, leading to multiform aneuploidy, haploidy, diploidy or triploidy, according to as the shock has set in at different times after the external fertilization. With the help of the hybrid *S. salar* × *S. trutta,* where the different haploid nuclei are distinguishable by their chromosome numbers, it was found that the haploids produced by the cold treatment were *maternal ones* (contrary to the hypotheses propounded earlier with regard to the *Amphibia*). The triploids appear by fertilization of diploid egg nuclei. In gwyniad, the haploids produced by the cold treatment may either have fragments, or lack them. From this, it is evident that the female is probably heterogametic in this species.

22. *The mitotic genes, i. e.* the genes regulating a normal mitosis, are of extreme significance with regard to the appearance of a sterility-barrier between different populations, and accordingly, also with regard to the evolution. The positions of the mitotic genes in the chromosome-set has been discussed, since the nuclei of *Coregonus* can accomplish mitosis with not more than 4 chromosomes. The internal balance in the mitoses activity, with an optimum of chromosomes which are multiples of 20, suggests that the Salmonoids are old polyploids.

23. A general survey of our present knowledge of the capacity of the chromosomes to perform morphological and numerical variations shows that phylogenetic »fusion» or »fragmentation» is a complicated process which cannot, conceivably, be pursued in ordinary, natural surroundings repeatedly in the same direction. A chromosome increase is most often connected with

the phenomenon called the ROBERTSON law. A chromosome increase, according to this principle, cannot explain the chromosome differences in the Salmonidae, which are grouped around the numbers 2 n = 60—80—100. On the other hand, this principle may account for the deviations from these basal numbers which have been noted. In order to throw light on the large chromosome differences 60—80—100 (which, moreover, do not correspond morphologically to the ROBERTSON law), another explanation has to be resorted to, *viz.*, polyploidization in the phylogeny. The basal number (x) must have been 10, whereupon it follows that the recent Swedish Salmonoids are hexa, octo- and decaploids.

24. The meiotic disturbances of the Salmonoids offer rather insignificant support to the theory of their polyploid origin but, do not on the other hand, gainsay it.

25. The systematic variation of the Salmonoids and their numerous forms, modifications, etc., constitute a variable wilderness for the taxonomists. This variation of their may be due to a comparatively lively re-arrangement of the gene material. This re-arrangement is most pronounced in the gwyniad, which also reveals the most pronounced variation and racial diffusion. The fairly great gene tolerance is a prerequisite with regard to the phylogenetic significance of the lively chromosome re-arrangement. This speaks in favour of the theory of polyploidization in phylogeny.

26. MULLER (1925, 1941) has pointed out the obstacles to be encountered with regard to the stabilization of polyploidy in the bisexual animal kingdom. A survey of the results hitherto obtained concerning the sex-determination of fishes shows that this mechanism is of a special kind. The genes of the heterogametic sex lie in Y, while X is devoid of sex realizators. Apart from these sex realizators, a number of polyfactorial genes cooperate, which are distributed in the genome and, in certain cases, they determine the sex without sex realizators. Two of MULLER'S obstacles are removed by this mechanism. The third and last one represents the difficulty, on the part at a spontaneous, polyploid animal, of »finding» a similarly polyploid partner, triploids being excluded as an intermediary stage in more than one generation. It has been suggested that these difficulties could have been got rid of by faunal deviations, accompanied by hybrid swarms with a high number of unreduced gametes.

27. The cases of polyploidy in animals, hitherto established, have been reviewed. VANDEL has listed a number of cases of chromosome multiples of animals which he believes to have occurred by simultaneous fragmentation of the whole chromosome set. This interpretation is repudiated as incompatible

with our present cytological knowledge. MULLER'S famous rule is based on a generalization of the sex-chromosome mechanism of the *Drosophila*. Many diocious plants have, as probably the majority of or all fishes, quite a different mechanism. In all likelihood, this mechanism is to be found also in other organism groups. When a very long time has elapsed since the occurrence of the polyploidization during the process of the evolution, there is nowadays in several cases no possibility of ascertaining this polyploidization. Scientists have concentrated on attempts to find the different multiples of one and the same basal number in a systematically delimited group. Accordingly, the conception that polyploidy lacks phylogenetic significance in the animal kingdom is probably more or less incorrect.

VII. Microphotographs.

Plate I.

1. Salmo salar. Secondary constrictions in prometaphase. 4 000 ×. 2. Coregonus lavaretus. Normal anaphase. Note: the variation in division of the centromeres. 3 000 ×. 3. Salmo salar. Mitotic metaphase. 60 chromosomes. 1 700 ×. 4. M I in polar view. 2 300 ×. 5. M I in side view. 2 300 ×. 6. M I in side view, showing univalents. 2 300 ×. 7. Salmo trutta. Mitotic metaphase. 80 chromosomes. 1 700 ×. 8. M I in polar view. 2 300 ×. 9. Salmo salar × Salmo trutta. Mitotic metaphase. 70 chromosomes. 1 700 ×. 10. M I in polar view. 2 300 ×. 11. M I in side view. Note: two distinct univalents. 2 300 ×. 12. Salmo trutta × × (Salmo salar × Salmo trutta). Normal metaphase plate, containing 75 chromosomes. 1 700 ×. 13. Multipolar anaphase. 1 700 ×. 14. Multipolar anaphase, the chromosomes unspiralized and pycnotic. 1 700 ×.

Plate II.

15. Salmo trutta × (Salmo salar × Salmo trutta). Multipolar anaphase with numerous fragment chromosomes. 1 700 ×. 16. Prometaphase containing 37 chromosomes. 1 700 ×. 17—20. Anaphase sticking. 1 700 ×. 21. Anaphase sticking leads to chromosome elimination. 1 700 ×. 22. Two chromosomes cannot orientate into the normal metaphase plane. 1 700 ×. 23. A giant chromosome in early anaphase is longer than half the spindle. Its centromere is just about to divide. 1 700 ×. 24. Salmo alpinus. Mitotic metaphase. 80 chromosomes. 700 ×. 25. Salmo trutta × Salmo alpinus. M I in side view. 2 300 ×. 26. Salmo fontinalis. Mitotic metaphase. 84 chromosomes. 1 700 ×. 27. Salmo fontinalis × Salmo trutta. M I in side view. 2 300 ×. 28. Coregonus lavaretus. Mitotic metaphase. 80 chromosomes. 700 ×. 29. Telophase-chromosomes after strong anaphase sticking. 1 700 ×.

Plate III.

30—31. Coregonus lavaretus. Unbalanced telophase. The centromeres of the longest chromosome, showing its chromatids still sticking together, has precociously divided. 1 700 ×. 32. Coregonus lavaretus. Anaphase. Two chromatid-pairs show typical non-disjunction, wandering to the same pole. 2 300 ×. 33. Detail of a late metaphase, the fragment is dividing precociously. 2 300 ×. 34. Coregonus albula. Mitotic metaphase. 80 chromosomes. 1 700 ×. 35. Unbalanced anaphase. Note the dicentric chromatids and the apparent anaphase sticking. 2 300 ×. 36. Dicentric chromosome in mitotic metaphase. One centromere is divided, the other not. 1 700 ×. 37. Thymallus thymallus. Mitotic metaphase. 102 chromosomes. 1 700 ×. 38. Osmerus eperlanus. Mitotic metaphase. 58 chromosomes. 1 700 ×. 39. Salmo alpinus. Tetraploid metaphase. 160 chromosomes. 1 700 ×. 40. Coregonus lavaretus. Octoploidization by colchicine treatment. Note the c-pairs. 1 700 ×. 41. Salmo alpinus. Spontaneous octoploidization. No spindle is developed 1 700 ×.

Plate IV.

42. Salmo trutta. Haploid metaphase. 40 chromosomes. 1 700 ×. 43. Triploid metaphase. 120 chromosomes. 1 700 ×. 44. Coregonus lavaretus. Cold treatment. Haploid metaphase. 1 700 ×. 45. Cold treatment. Metaphase with 28 chromosomes. 1 700 ×. 46. Cold treatment. Metaphase with 7 chromosomes. 1 700 ×. 47. Cold treatment. A 4-chromosome-nucleus at anaphase. 1 700 ×. 48. Coregonus albula. Metaphase, showing ring-chromosomes, giant chromosomes and fragments. 2 300 ×.

No microphotographs have been retouched.

Plate I.

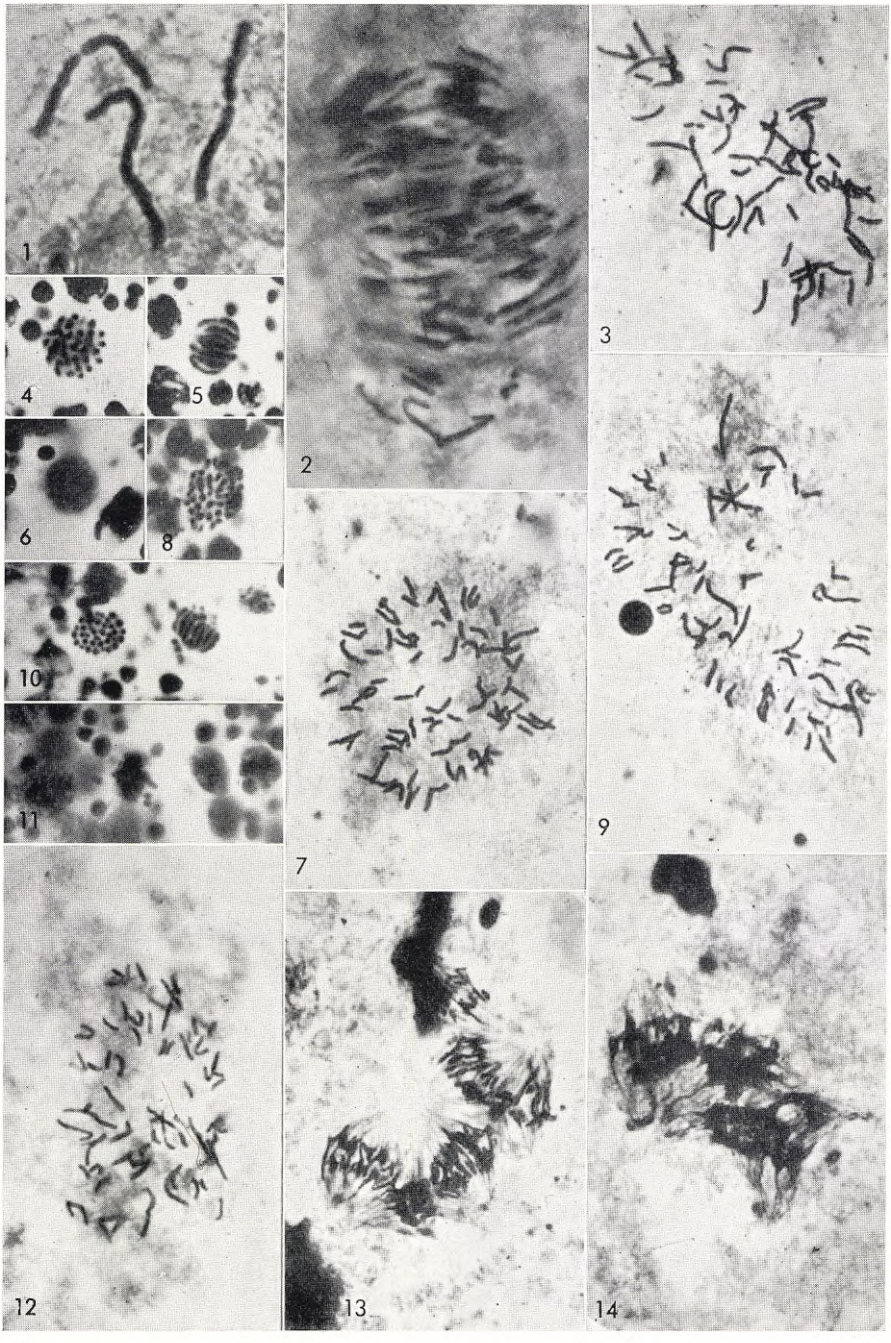


Plate II.

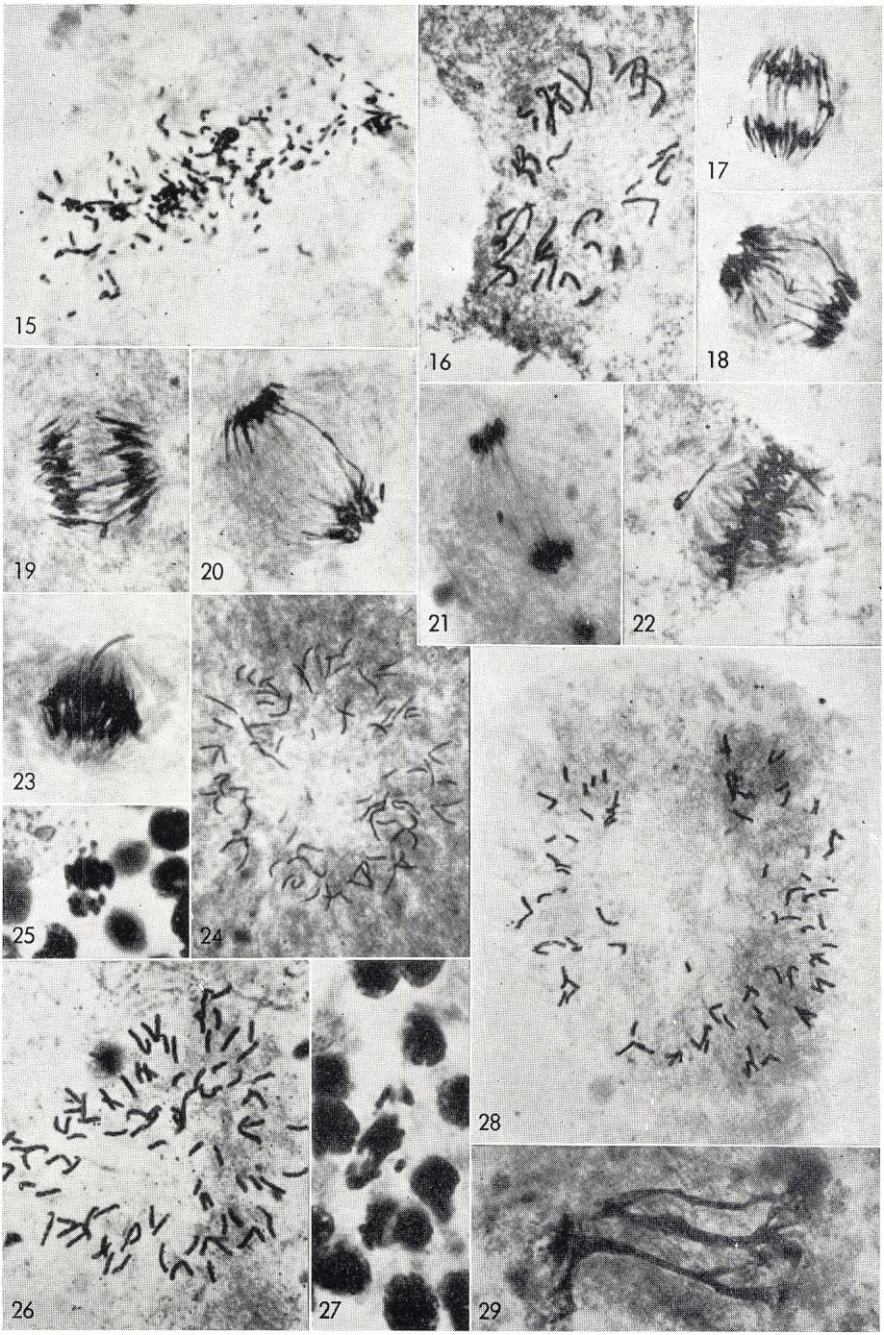


Plate III.

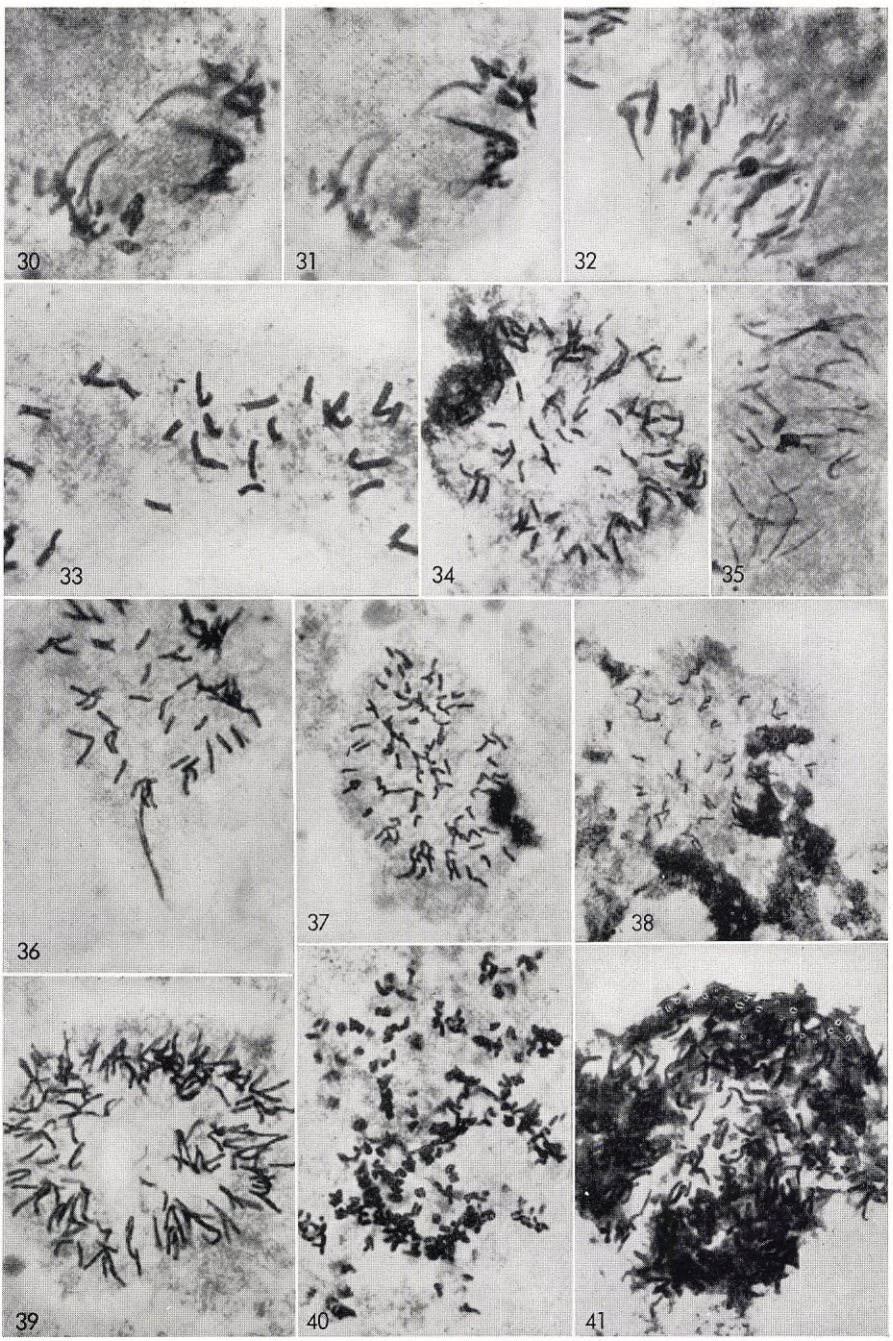
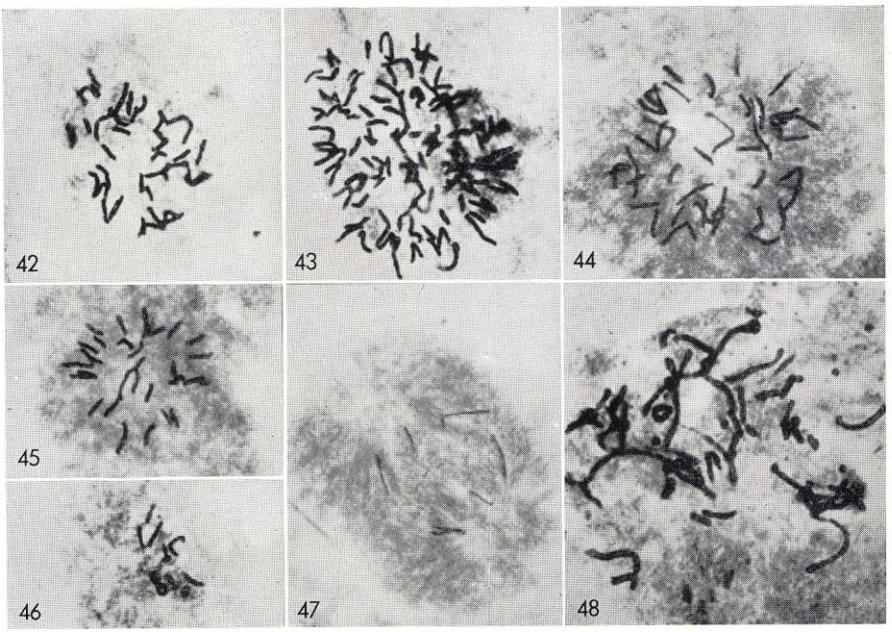


Plate IV.



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