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CHROMOSOMA

The Diminution of Heterochromatic Chromosomal Segments in *Cyclops* (Crustacea, Copepoda)

Sigrid Beermann

Max-Planck-Institut für Biologie, Spemannstr. 34, D-7400 Tübingen,
and Institut für Genetik, Universität Hohenheim, D-7000 Stuttgart 70, Federal Republic of Germany

Abstract. The chromosomes of *Cyclops divulsus, C. furcifer,* and *C. strenuus,* like those of several other Copepods, undergo a striking diminution of chromatin early in embryogenesis. The process is restricted to the presumptive soma cells and occurs at the 5th cleavage in *C. divulsus,* at the 6th and 7th in *C. furcifer,* and at the 4th in *C. strenuus.* The eliminated chromatin derives from the excision of heterochromatic chromosome segments (H-segments). Their chromosomal location is different in the three investigated species: Whereas in *C. divulsus* and *C. furcifer* the H-segments form large blocks – exclusively terminal in the former and terminal as well as kinetochoric in the latter – the germ line heterochromatin in *C. strenuus* is scattered all along the chromosomes. Extensive polymorphism exists with respect to the length of the terminal H-segments in *C. furcifer,* and with respect to the overall content of heterochromatin in the chromosomes of *C. strenuus.* In a local race of *C. strenuus* an extreme form of dimorphism has been found which is sex limited: females as a rule are heterozygous for an entire set of large (heterochromatin-rich), and a second set of small chromosomes in their germ line. Males are homozygous for the large set. In the first three cleavage divisions the H-polymorphism is solely expressed through differences of chromosome length. Following diminution the differences between homologous have disappeared. Feulgen cytophotometry demonstrates that in the three species the 1C DNA value for the germ line, as measured in sperm, is about twice that measured in somatic mitoses (germ line/soma C-values in picograms of DNA: *C. strenuus* 2.2/0.9, *C. furcifer* 2.9/1.44, *C. divulsus* 3.1/1.8). – The data imply that chromatin diminution is based on a mechanism which allows specific DNA segments, regardless of their location and size, to be cut out from the chromosomes without affecting the structural continuity of the remaining DNA. This mechanism may be analogous to that of prokaryotic DNA excision.

A. Introduction

Extra heterochromatic material as a constituent of the genome has been found in a variety of plants and animals either in the form of supernumerary entire

chromosomes or as extra chromosome segments. In both situations a distinction can be made between such elements which are present in all cells of an organism and those which, as a consequence of somatic elimination, remain limited to the germ line. Supernumerary chromosomes not limited to the germ line are known as B-chromosomes (White, 1954; John and Lewis, 1968). In cases where there is germ line limitation they have been designated either as E-chromosomes (Cecidomyiidae), K-chromosomes (Chironomidae) or as L-chromosomes (Sciaridae).

Extra chromosomal segments are as a rule recognized as unequal bivalents so that in heterozygotes one homologue exhibits a segment which is absent in the other. In all instances hitherto studied, such extra chromosomal segments have been found to be heterochromatic. Their mitotic and meiotic behavior is normal, and they are generally present in both soma and germ line (cf. White, 1973). In Ascarids (Nematoda) and in many Copepods, however, certain heterochromatic chromosomal segments are eliminated from all of the presumptive somatic cells during the early stages of embryogenesis and are retained only in the germ line. In *Ascaris megalocephala* (=*Parascaris equorum*) the two or four chromosomes (so-called Sammelchromosomen) break apart into 52 to 72 (*var. univalens*), single elements during the second through fifth cleavage divisions, and in the process of this breakdown the terminal heterochromatic segments are discarded ("chromatin diminution" Boveri, 1887). In *Ascaris lumbricoides*, according to Bonnevie (1901), the heterochromatic terminal segments are removed in the same way as in *A. megalocephala*, but the remaining intermediate segments do not undergo fragmentation.

In Copepods, an elimination of chromatin in all primordial cells of the soma has been observed in the following species: *Cyclops divulsus, C. furcifer, C. strenuus, C. varicans,* and *Canthocamptus staphylinus* (S. Beermann, 1959), and also in *C. bohater, C. vicinus* (Einsle, 1964) and *Acanthocyclops vernalis* (Akifjev, 1974). This phenomenon was either not recognized by previous authors, or misinterpreted as pathological (Häcker, 1894; Amma, 1911) or as synthesis of extra DNA (Stich, 1954, 1962). The process of chromatin diminution in Copepods essentially resembles that in *Ascaris*. The Copepods, however, afford much better conditions for a detailed cytological analysis of behavior and distribution of the germ line limited chromatin during all phases of the life cycle. The diminution of the heterochromatic segments in *Ascaris* has generally been understood as a breaking off of terminal chromosome pieces (e.g., Beams and Kessel, 1974). However, a preliminary investigation of *C. furcifer* (S. Beermann, 1966) clearly indicated that in Copepods the diminution mechanism is more complicated and that diminution can take place irrespective of where the extra heterochromatic segments are situated within the chromosomes (Fig. 1). The analysis of the chromosome cycle of *C. strenuus* reported in the present study affords further and even more dramatic evidence that interstitial as well as terminal chromosome segments can be eliminated.

B. Materials and Methods

a) Taxonomic and Ecological Data. The Copepods studied in this paper, *Cyclops furcifer* Claus, 1857 (=*C. furcifer furcifer* Lindberg, 1957), *C. strenuus strenuus* (=*C. rubens rubens* Lindberg, 1957)

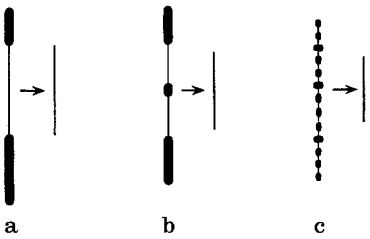


Fig. 1a–c. Schematic drawing of a typical chromosome from
a *C. divulsus,*
b *C. furcifer,*
c *C. strenuus,*
with H-segments prior to diminution, and without them after the diminution

and *C. strenuus divulsus* Lindberg, 1957 (=*C. abyssorum divulsus* Einsle, 1964) belong to the genus *Cyclops s. str.* This genus comprises at least seven full species, some of which have been divided into a number of subspecies, races, or varieties, all in all more than 52 described forms (Kiefer, 1927, 1954; Lindberg, 1957; Einsle, 1975). The members of the genus *Cyclops s. str.* were earlier regarded as all belonging to the same species, *Cyclops strenuus.* The three species which hereafter will be simply designated as *C. divulsus, C. furcifer* and *C. strenuus* inhabit stagnant ponds and puddles which often dry out during the summer. The individuals used in this study were taken from the following localities, all except MA in Württemberg:

C. divulsus from Pfronstetten (PF) near Zwiefalten and from Seehof (SE) near Hechingen; *C. furcifer* from Marburg (MA), Reusten (RI) and Buchhöfe (BU) near Horb; *C. strenuus* from the pond near Seehof (SE), Hohentringen (HO) and Reusten (R2) and (R3).

b) Laboratory Culture. The culture conditions worked out by Spindler (1971) for *C. vicinus* (light: darkness=16 h:8 h, light intensity 200 lux, temperature of 20° C, pH of 6.45) were also tested for *C. divulsus* and *C. furcifer.* The animals were fed with *Haematococcus pluvialis.* In *C. divulsus* all of the nauplii hatched 4–6 days after oviposition. After 10–12 days most of them had developed into copepodids, and adults appeared after 18–22 days. The rearing of the nauplii and the first copepodid stage did not present problems, but among the 4th and 5th instar copepodids as well as the young adults the mortality was very high. On the average only 20% of the progeny developed into mature adults. In breeding experiments with *C. furcifer* almost all of the animals died after 18–20 days, but in some dishes 20% survived. These animals could be kept at 4° C for a number of months, from the end of April until the beginning of October.

c) Preparation of Slides. In order to obtain slides with meiotic divisions, whole animals were fixed in alcohol-glacial-acetic acid (3:1). The gonads were dissected in 50% acetic acid, stained with lactic-acetic-orcein and then squashed with moderate pressure. To analyze the cleavage divisions, a few eggs were separated from freshly deposited egg batches after appropriate intervals and then fixed in alcohol-acetic acid, stained in a small amount of lactic-acetic-orcein and then gently squashed by cautiously placing a coverslip on them.

d) Feulgen Cytophotometry. Developing embryos together with sperm were fixed in alcohol-acetic acid 3:1 and squashed in 50% acetic acid on extra thin slides (0.7 mm). After no more than 5 min slides were frozen on dry ice and the cover slips pried off. The preparations were immediately placed in isopropanol where they remained for 8–10 days at 4° C. Chick blood was diluted with 4% sodium citrate, spread on some of the slides carrying the *Cyclops* material. All of the slides were subjected to a second fixation treatment in ethanol:formol=4:1 for 30 min; they were hydrolyzed 60 min in 5 N HCl at 25° C (De Cosse and Aiello, 1966; Rasch and Rasch, 1973), and afterwards immersed in Schiff's reagent, which had been prepared from acridine-free Pararosaniline (Chroma IB 533) 1 d before staining. After repeated washes in distilled water and SO_2-water, all of the slides were passed through the alcohol series into xylol, then embedded in Caedax and allowed to dry in the dark for 10 d at room temperature.

The DNA of the Feulgen stained slides was measured cytophotometrically with the UMSP 1 (Carl Zeiss) at a wave length of 560 nm, and with an object advance speed of 40 μm/min and a diaphragm measuring 1 μm in diameter. The lines scanned were 1 μm apart.

C. Results

I. Survey of the Cytology and Early Development of Cyclops s. str.

1. Chromosome Complement

As first reported (Häcker, 1890, 1892; Braun, 1909; Matschek, 1910), the species of the genus *Cyclops s. str.*, which were previously considered to constitute the species "*C. strenuus*", all have 2n=22 chromosomes. The chromosomes are meta- to submetakinetic. Sex chromosome dimorphism has never been observed. Conspicuous cytological differences between the species exist in the location and distribution of heterochromatic segments in the germ line chromosomes and in the mode of heterochromatin diminution. These will be considered in detail in the species *C. furcifer* and *C. strenuus* (compare S. Beermann, 1959; Einsle, 1964, 1975).

2. Oogenesis and Spermatogenesis

The structure of the female and male gonads in Copepods and the cytology of meiosis were described at the turn of the century by a number of authors (Häcker, 1895, 1902; Lérat, 1905; Matschek, 1910). The earliest recognizable meiotic stage is pachytene. At the beginning of the growth phase of the oocyte, the bivalents enter into diplotene, the pairing split becoming conspicuously wider. The chromosomes assume a lampbrush structure. At mid diplotene, the lampbrush bivalents attain their greatest length. Late diplotene coincides with the end of the growth phase and is characterized by a gradual contraction of the bivalents, which finally ends in a typical diakinesis (prometaphase, Rüsch, 1960). Throughout diakinesis the bivalents remain distance-paired, often separated by a considerable gap, and in this condition enter the equatorial plane of the first division spindle. The homologues are always oriented parallel to each other, and no recognizable chiasmata are ever formed between them. Oogenesis is probably achiasmatic in all Copepods. A distance pairing such as is described here has also been found in other cases of achiasmatic meiosis.

The spermatogenesis of Copepods was studied by Häcker (1902), Lérat (1905), Chambers (1912), Kornhauser (1915) and especially Heberer (1924, 1932, 1938). Meiosis in the male is probably of the chiasmatic type, but it is difficult to demonstrate this in detail (Heberer, 1938; see also p. 323 of this paper).

3. Early Development

a) General Remarks. The early development of Copepods, especially of Cyclopids, has been the subject of several classical studies (Häcker, 1892; Rückert, 1895; Amma, 1911). The mitotic configurations of the cleavage divisions are unusually clear as a consequence of the relatively large size of the chromosomes and the separation of maternal and paternal sets in the spindle, known as

gonomery (Häcker, 1896). Since chromatin diminution in Copepods takes place during the early development, the sequence of cleavage divisions in the three species which are the primary objects of this study, *C. divulsus, C. furcifer,* and *C. strenuus,* will be described in detail.

As in all Copepods the development is strictly determinate. Already in the first cleavage divisions stem cells for certain embryonic anlagen are segregated (Häcker, 1897, 1903; Amma, 1911). This is especially true for the differentiation between soma and germ line. Häcker observed an accumulation of granules (ectosomes) in the proximity of one of the spindle poles during the first cleavage division in the species *C. viridis* and *Diaptomus denticornis.* These ectosomes continue to be transmitted to a single cell during the following cleavages. As indicated by Amma (1911) this type of ectosome transmission is repeated up to the 6th cleavage division, and he was able to show that the original ectosome-containing cell gave rise to the two primordial germ cells. At least during the first three cleavage divisions the development of *C. divulsus, C. furcifer* and *C. strenuus* parallels that which Rückert (1895) and Amma (1911) described for "*C. strenuus*". Following the union of the pronuclei the first cleavage division occurs at about 1 h after oviposition (at 20° C). The next divisions follow at intervals of from 45–60 min. Mitosis as such, i.e., the time from prophase to telophase takes only about 15 min. Until the 8-cell stage all of the divisions are synchronous. After that, specific asynchronies occur, depending on the species.

b) C. divulsus. The blastomeres of *C. divulsus* divide synchronously until the end of the 4th cleavage division (16-cell stage). The interphase following this cleavage lasts 3–4 h, and is thus significantly longer than the preceding ones. At this time the heterochromatic nature of the chromosome ends becomes apparent, and 2 large nucleoli are formed. Of the 16 cells resulting from the 4th cleavage, one divides again after 60–90 min. One of the two daughter cells from this division is the progenitor of the two definitive primordial germ cells (PGC), and the other is believed to be the stem cell of the entoderm (PEC) (Amma, 1911). Both cells do not participate in the 5th cleavage division, during which the diminution mitosis takes place in the other 15 cells (S. Beermann, 1959). In this manner 32 cells are formed, 30 diminuted cells + 1 PGC + 1 PEC. In a delayed division (more or less at the time of the 6th cleavage division of the other cells) the stem cell of the entoderm undergoes diminution, while the stem cell of the germ line divides without diminution to form the two definitive primordial germ cells (Fig. 2 and Table 1).

c) C. furcifer. In this species, as in *C. divulsus,* the cleavage divisions all occur synchronously up to the 16 cell stage. However, the interphase directly before the 5th cleavage lasts only 2–2.5 h. During this time one cell divides before the others so that here too only 15 of the 17 cells enter the 5th cleavage division (15 + 1 PGC + 1 PEC). However, chromatin diminution does not yet take place, although the heterochromatic portions of the chromosomes have already become quite distinct. They are located at the ends of the chromosomes and at the kinetochores. The 5th cleavage division, therefore, gives rise to

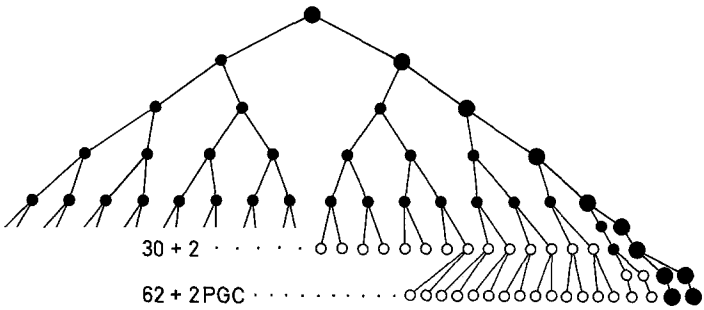


Fig. 2. Cleavage and diminution program in *C. divulsus*: ● = not diminuted (germ line large circles), ○ = diminuted, PGC = primordial germ cell

Table 1. Time table of cleavage divisions in *C. divulsus* at 18–20° C

Cleavage interval	Duration of interval in min	Number of nuclei		Special features
		at beginning of division	at end of division	
0–1	45– 60	1	2	No differentiation between eu- and heterochromatin
1–2	45–60	2	4	No differentiation between eu- and heterochromatin
2–3	45–60	4	8	No differentiation between eu- and heterochromatin
3–4	45–60	8	16	No differentiation between eu- and heterochromatin
4–5	210–240	15+1 PGC +1 PEC	30+1 PGC +1 PEC	Differentiation into eu- and heterochromatin. After 60 min division of 1 cell, formation of nucleoli. Diminution at 5th cleavage in 15 cells
5–6	45– 60	30+2 PGC +2 PEC	62+2 PGC	Division of the PGC between 5th and 6th cleavage without diminution. Diminution division of the PEC. Heterochromatin only in 2 PGCs

30+1 PGC+1 PEC=32 not diminuted nuclei. A first diminution takes place during the 6th cleavage division in 30 cells. Subsequently one of the two remaining cells divides without diminution, giving rise to the two primordial germ cells (PGC). This division can be delayed until the 7th cleavage which begins with 60 diminuted cells+2 PGC+1 PEC=63 cells. At first, the cells which have completed the first diminution undergo a second diminution of chromatin. The primordial entoderm cell and its 2 daughter cells are diminuted when the rest of the soma cells have completed two more divisions. In 28 embryos studied during the 9th cleavage division each had 2 nuclei with thick chromocen-

ters. These represent the 2 primordial germ cells which divide only at late larval stages. The sequence of events during the cleavage divisions of *C. furcifer* is summarized in Table 2 and Figure 3.

d) C. strenuus. In this species the division of one of the blastomeres is already delayed during the 3rd cleavage division. According to Amma and Häcker, this cell represents the stem cell of the germ line. Prior to the 4th cleavage division, there is an unusually long interphase which lasts 7–8 h in 7 of the 8 blastomeres. The 8th blastomere, which is perhaps derived from the late dividing cell mentioned above, completes a mitosis in advance of the other cells. One of its two daughter nuclei remains in a telophase-like state with condensed chromosomes. This, as can be seen from its further development, is the precursor of the primordial germ cell. The other daughter nucleus, like the rest of the blastomeres, passes into a pale-staining, decondensed interphase stage. It is considered either as stem cell of the entoderm, or as giving rise to it. Only 7 of the 9 cells in each embryo participate in the 4th division step which is accompanied by chromatin diminution already at this relatively early stage so that the 4th cleavage ends with 14 diminuted cells + 1 PGC + 1 PEC = 16 cells. However, while the 4th cleavage is still in progress, or immediately afterwards, the PGC divides without diminution (Fig. 30). Their daughter cells are the 2 definitive PGCs. The total cells number now is increased to 17. The cell believed to be the PEC undergoes diminution in the 5th cleavage (Fig. 31). The 5th cleavage division, therefore, terminates with 32 cells (28 + 2 PGC + 2 PEC). Both of the undiminuted primordial germ cells, whose nuclei can be easily recognized by the unusual distribution of their chromatin remain undivided until late stages of development (Fig.31).

In *C. strenuus* (R3) sister embryos from the same batch show striking differences in the rate of their development depending upon whether their karyotype is homozygous or heterozygous for the set of heterochromatin-rich chromosomes (see p. 325). Already at the 3rd cleavage, the division of homozygous eggs is delayed as compared to that of the heterozygous ones (Table 4). Again at the 4th cleavage step, division is found to be well underway in some eggs, while other still remain in the long interphase. Subsequently, the difference becomes even more pronounced so that, when some of the eggs have already completed the 5th cleavage, others are only at the 4th one. This variability makes it difficult precisely to define the duration of the interphase between the 4th to 5th cleavage divisions. The sequence of cleavage events is summarized in Table 3 and Figure 4.

II. Structure of Chromosomes and Chromatin Diminution in C. furcifer

1. Distribution and Polymorphism of Germ Line Heterochromatin

a) Oogenesis. The chromosomes in the germ line of *C. furcifer* are characterized by long heterochromatic segments (=H-segments). Unlike the situation in *C. di-*

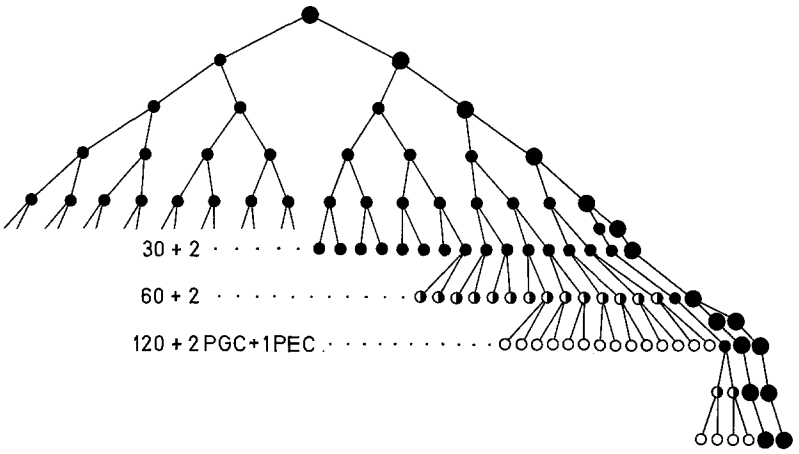


Fig. 3. Cleavage and diminution program in *C. furcifer*: ● = not diminuted, ◑ = after the 1st diminution, ○ = diminuted, PGC = primordial germ cell, PEC = primordial entoderm cell

Table 2. Time table of cleavage divisions in *C. furcifer* at 18–20° C

Cleavage interval	Duration of interval in min	Number of nuclei		Special features
		at beginning of division	at end of division	
0–1	45– 60	1	2	No differentiation between eu- and heterochromatin
1–2	45– 60	2	4	No differentiation between eu- and heterochromatin
2–3	45– 60	4	8	No differentiation between eu- and heterochromatin
3–4	45– 60	8	16	No differentiation between eu- and heterochromatin
4–5	120–150	15 + 1 PGC + 1 PEC	30 + 1 PGC + 1 PEC	Differentiation into eu- and heterochromatin. After 60 min division of 1 cell, formation of nucleoli
5–6	45– 60	30 + 1 PGC + 1 PEC	60 + 1 PGC + 1 PEC	1st diminution at 6th cleavage in 30 cells
6–7	45– 60	60 + 2 PGC + 1 PEC	120 + 2 PGC + 1 PEC	Division of the PGC between 6th and 7th cleavage without diminution. 2nd diminution at 7th cleavage
7–8	45– 60	120 + 2 PGC + 2 PEC		Diminution division of the PEC after the 7th and the 8th cleavage. Heterochromatin only in 2 PGCs

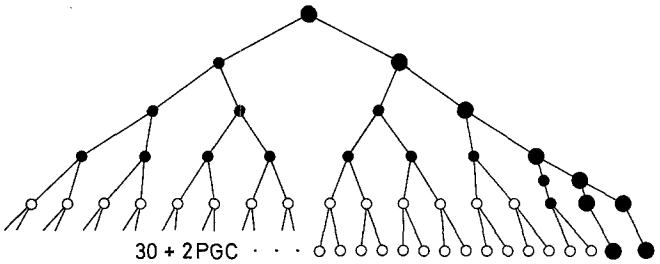


Fig. 4. Cleavage and diminution program in *C. strenuus*: ● = not diminuted, ○ = diminuted, PGC = primordial germ cell

Table 3. Time table of cleavage divisions in *C. strenuus* at 18–20° C

Cleavage interval	Duration of interval in min	Number of nuclei		Special features
		at beginning of division	at end of division	
0–1	45–60	1	2	No differentiation between eu- and heterochromatin
1–2	45–60	2	4	No differentiation between eu- and heterochromatin
2–3	45–60	4	8	No differentiation between eu- and heterochromatin
3–4	7– 8 h	7+1 PGC +1 PEC	14+2 PGC +1 PEC	Precocious division of 1 cell. Formation of nucleoli. Diminution division at 4th cleavage in 14 cells. Division of the PGC without diminution
4–5		14+2 PGC +1 PEC	28+2 PGC +2 PEC	Diminution division of the PEC at 5th cleavage. Heterochromatin only in 2 PGCs

vulsus (S. Beermann, 1959), all 11 chromosomes in *C. furcifer* exhibit a block of heterochromatin next to their kinetochores in addition to long terminal H-segments. This is especially apparent during the late prophase stages of oogenesis.

In the initial stages of oocyte development the chromatin appears as fused into a single dark staining block (Fig. 5a). This stage must correspond to leptotene and zygotene since in oocytes with somewhat larger nuclei, segments of pachytene bivalents can already be discerned (Fig. 5b). As the oocyte grows, the blocks of heterochromatin begin to become disentangled. The chromosomes stretch out, and the blocks of kinetochore heterochromatin appear as isolated, paired chromocenters (Fig. 5c). This marks the beginning of diplotene. In the subsequent stages of diplotene the bivalents gradually become identifiable over their entire length (Fig. 6). The pairing split in the kinetochoric heterochromatin becomes increasingly distinct, and the terminal H-segments despiralize further. At the stage when the oocyte nuclei have attained their largest diameter, i.e.,

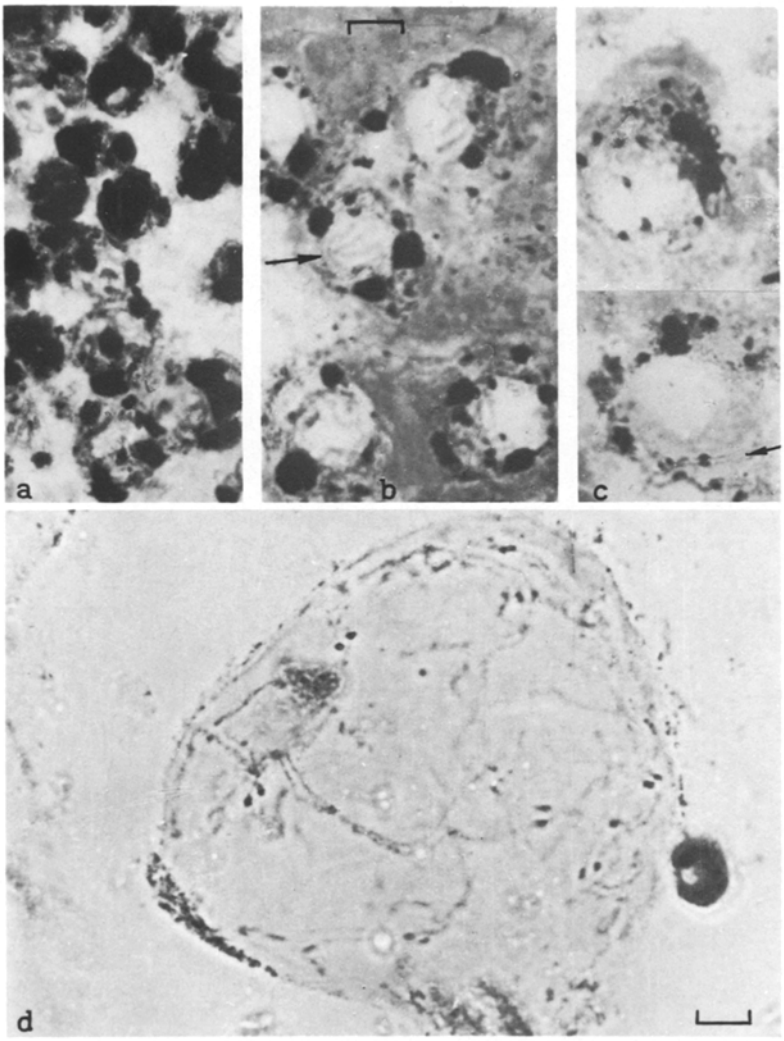


Fig. 5a–d. *C. furcifer*, meiotic prophase in oocytes: **a** Earliest stage: chromatin condensed into a dark staining block. **b** Early and **c** late pachytene. Euchromatin appears as pale staining strands (arrow). Terminal H-segments still fused. **d** Oocyte nucleus towards end of the growth phase: all of the heterochromatin is decondensed, except for the paired, dot-like, kinetochore segments. In this and the following illustrations the bar equals 10 μm, if not otherwise stated

approximately 8 times that of nuclei at the earliest prophase stages, the bivalents are maximally extended, and the euchromatic regions have assumed lampbrush structure. The homologues are distance-paired in the manner typical of achiasmatic meiosis. The terminal H-segments sometimes do not pair and spread apart. A distinct chromatid split appears in them. Whereas the H-segments at the kinetochore remain heteropycnotic during this stage, the extremely stretched terminal segments in both homologues temporarily assume a loosened, lampbrush-like, structure, similar to that of euchromatin (Fig. 6). The contrac-

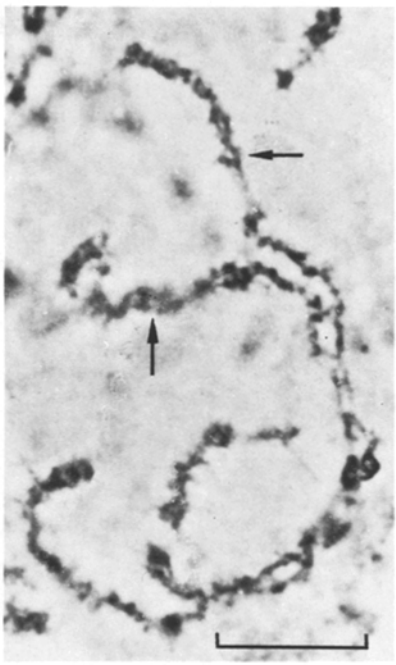


Fig. 6. *C. furcifer*, diplotene bivalent with lampbrush structure also in the terminal H-segments. The H-segments are unpaired, spread apart, and have already split into chromatids (arrow). Kinetochoric segments heteropycnotic

tion of the diplotene bivalents in the second half of oocyte growth begins at the terminal H-segments while the euchromatic regions retain their lampbrush structure for some time (Fig. 7). As a result, eu- and heterochromatin are quite easy to tell apart at this stage (Figs. 8–10), and it becomes apparent that length differences between homologues are solely due to the different lengths of their terminal H-segments. Towards the beginning of diakinesis the kinetochoric H-blocks become indistinct. Finally, in metaphase I, the terminal H-segments also can no longer be distinguished. Although the distribution of the H-segments in all of the 11 bivalents of *C. furcifer* follows the same general pattern, there is a considerable divergence between individual chromosomes especially in relation to the size of the terminal H-segments. A characterization of the 11 bivalents on this basis is, however, not possible since, as will be seen, the differences between both homologues of a pair are often greater than those between different bivalents. The euchromatic segments of all of the chromosomes, on the other hand, are very similar to one another so that structural criteria such as the ratio of the arm lengths cannot be applied. Only the nucleolar chromosome is easy to tell apart, since one of its arms bears a terminal nucleolus and, directly proximal to it, two smaller, intercalary H-segments (Figs. 7–10).

The diplotene karyotype has been found to be the same in all oocytes of a given individual, but it varies considerably between different members of the population. Therefore, the differences between homologues must be interpreted as representing structural heterozygosity, resulting from a genetic polymorphism of the heterochromatin. The frequency of heterozygotes in the popula-

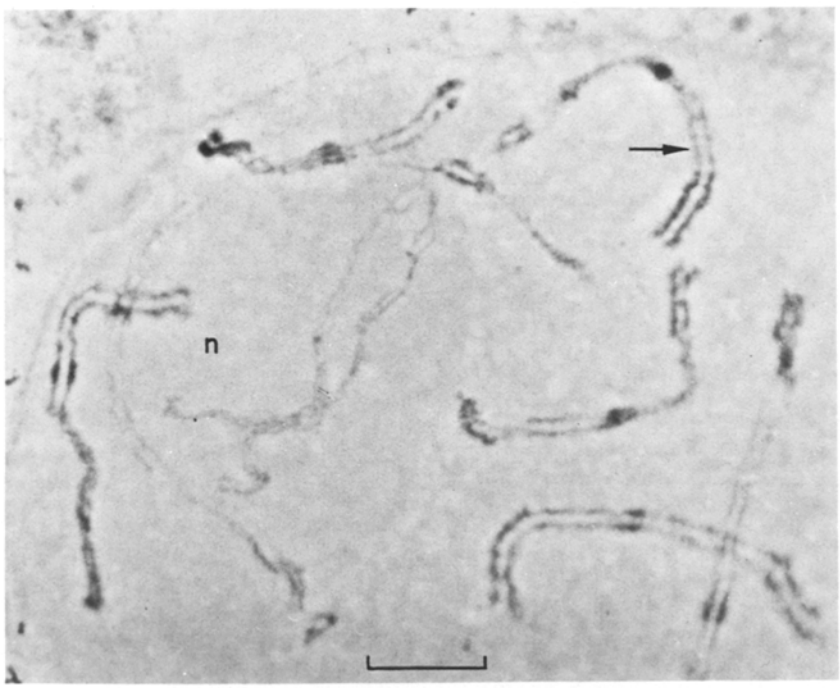


Fig. 7. *C. furcifer*, portion of oocyte nucleus showing 7 diplotene bivalents. Recondensation of terminal H-segments. Nucleolar chromosome with homozygous right arm (arrow) and despiralized nucleolus organizer terminating the left arm. Nucleolus (*n*) displaces a stretched-out bivalent towards the left

tion is very high, and so is the extent of heterozygosity within the karyotype. In 27 individuals, for example, 6 had 7 conspicuously unequal bivalents (see BU/9 in Fig. 9c), six had 6 unequal bivalents, and fifteen had 5 unequal bivalents (see BU/4 in Fig. 9b). None of the females was completely homozygous. The heterozygosity is not confined to only one arm of a given chromosome, as shown in Figure 9. In this figure, the first 5 or 6 chromosomes and the nucleolar chromosome form bivalents with typical structural heterozygosities. In the 4th bivalent from the left one of the homologues is conspicuous in not possessing a terminal H-segment on one of its arms (see also Fig. 1b in S. Beermann, 1966). Among 20 individuals in which all of the chromosome ends were clearly analyzable, 9 had two bivalents each with this extreme type of heterozygosity (Fig. 9c), whereas 10 individuals had only one (Fig. 9a, b). A few females had bivalents homozygous for the absence (or almost complete absence) of H-segments on one end (Fig. 11). From these observations, it could appear as if heterozygosity tended to occur always in the same pairs of chromosomes. However, as pointed out above, the identification of individual bivalents remains a problem even in cases of extreme heteromorphism, with the exception of the nucleolar bivalent. In this bivalent, only two different types of second arms are found in the population *C. furcifer* (BU) and (R1) (Fig. 10). Among 22 individuals of *C. furcifer* (BU) there were 14 heterozygotes and 8 homozygotes.

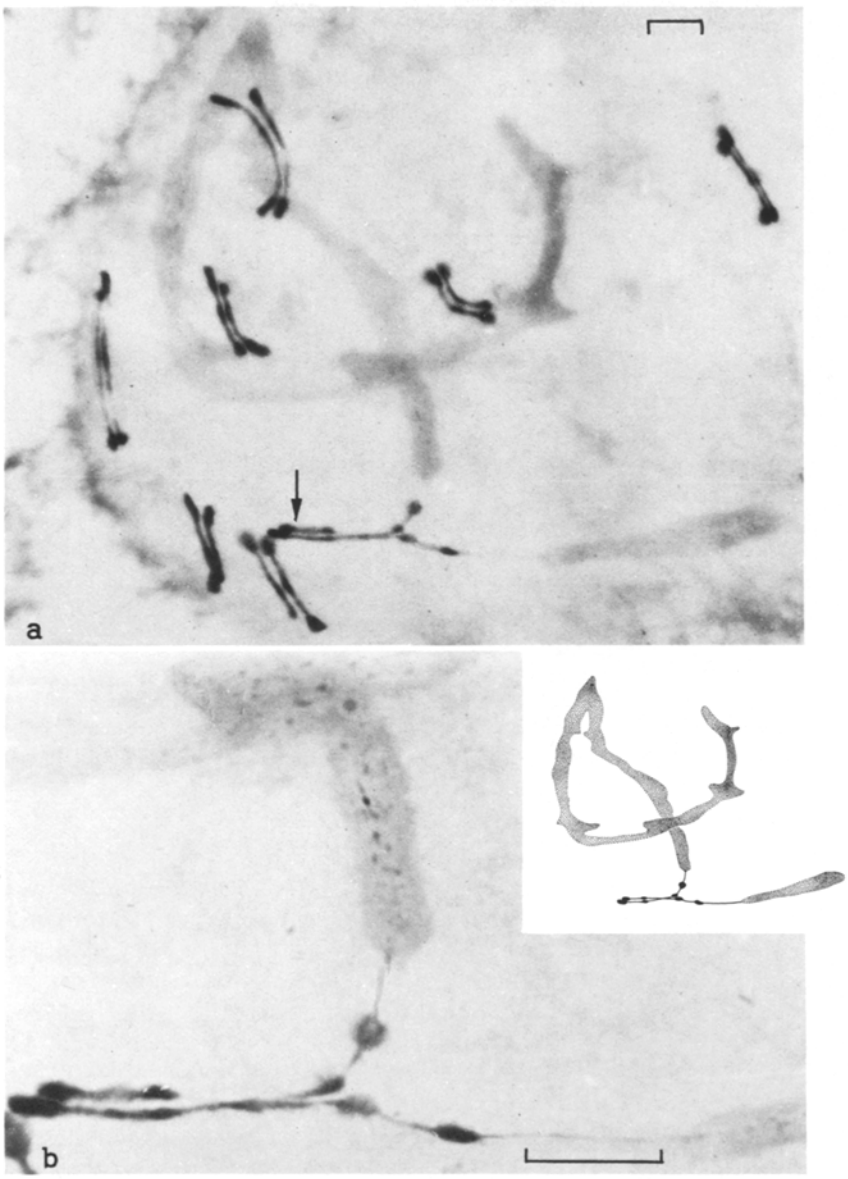


Fig. 8a and b. *C. furcifer,* late diplotene of oocyte: **a** Portion of a nucleus showing 8 bivalents. Terminal and kinetochoric H-segments well defined. Nucleolar chromosome (arrow) heterozygous for nucleolus organizer and for H-segments in the second arm. Giemsa-staining. **b** Nucleolar chromosome from **a**. The extremely stretched heterozygous nucleolar organizers form the axis of one long, and one short sausage shaped nucleolus. See diagram. Giemsa-staining

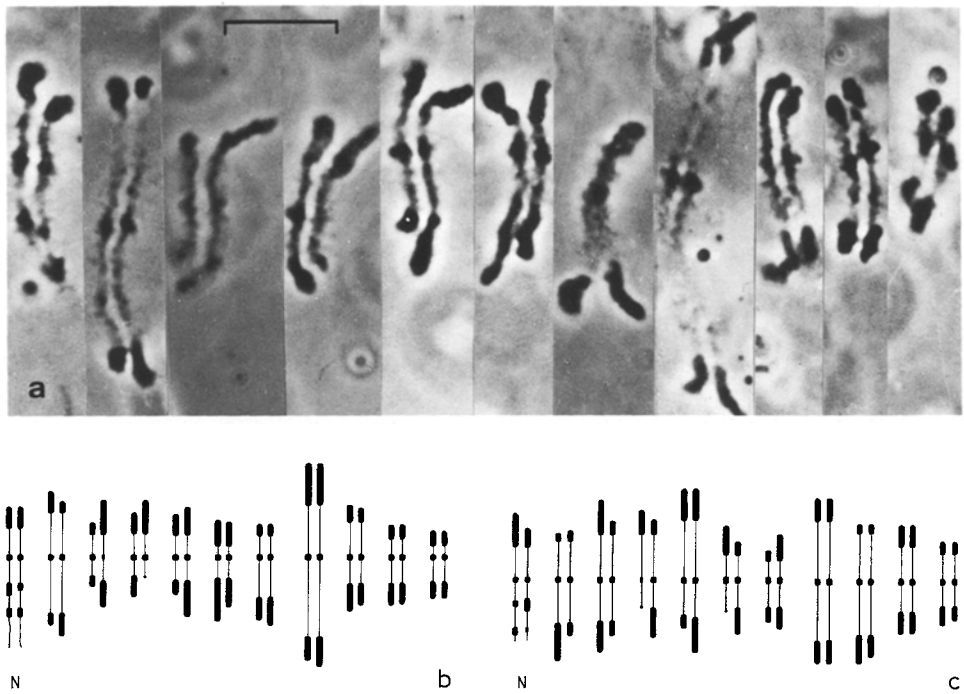
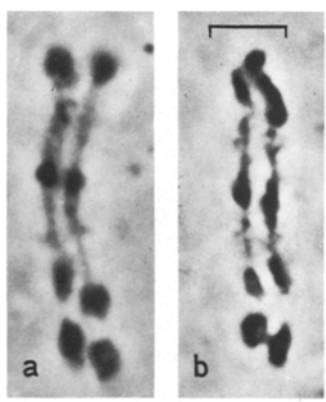


Fig. 9a–c. *C. furcifer*, distribution of heterochromatin at diplotene in oocytes: **a** Diplotene bivalent from an individual of the population Buchhöfe (BU/4). Left the nucleolar chromosome and 5 (probably 6) other unequal bivalents. **b** Schematic diagram of the diplotene karyotype shown in **a**. **c** Diplotene karyotype from another female (BU/9)



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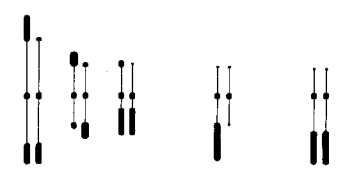

Fig. 10a and b. *C. furcifer*, nucleolar bivalents in diplotene. Upper end of chromosome with homozygous (**a**) or heterozygous (**b**) H-segments. In addition to the kinetochoric H-Blocks there are two other intercalated H-segments in the nucleolar arm. Terminal nucleolus organizer not apparent. Bar equals 5 μm

Fig. 11. *C. furcifer*, unusual distribution of heterochromatin in some bivalents from 3 individuals of the population Buchhöfe

There are indications that a polymorphism may also exist in intercalary H-segments. This is evident in the case of the nucleolar chromosome when comparing Figures 8–10.

b) Early Cleavage Divisions. During the first cleavage stages in *C. furcifer* as in *C. divulsus*, it is strikingly apparent that the chromosomes are not at all differentiated into eu- and heterochromatin. Chromocenters are not formed during the interphase, and during mitotic divisions the chromosomes are isopycnotic along their entire length. In both *C. furcifer* and *C. divulsus*, this condition persists until the end of the 4th cleavage division. Only after this division chromocenters appear in all of the 16 daughter nuclei. As seen in the subsequent 5th division, they correspond to the terminal and kinetochoric H-segments already known from the study of the diplotene bivalents in oogenesis. Especially during the prophase it can be readily seen that each chromosome has, in addition to its terminal H-segments, a smaller H-segment in the kinetochore region (Figs. 12b–14). The kinetochore is either enclosed within this segment or lies adjacent to it (Fig. 14). The segments which appear heterochromatic during meiosis and the 5th cleavage division must correspond to isopycnotic segments in the 1st to 4th cleavages (Fig. 12). In other words, the H-polymorphism shown to exist in diplotene chromosomes should be reflected in length differences between homologues during the early cleavage mitoses. The separation between paternal and maternal sets of chromosomes (gonomery) facilitates the demonstration of such differences. Thus, in trying to arrange the members of one set with those of the other in matching pairs, significant length differences between the homologues should be revealed. Such an analysis had already been carried out in *C. divulsus*. In *C. furcifer* the results are, in principle, identical. The analysis was performed by photographing suitable 4th cleavage anaphases in three different focal planes. The individual chromosomes of both sets were then drawn, and, as far as possible, arranged into matching pairs (Fig. 15a, b). Four anaphases, taken from different slides, were analyzed; in each of them at least three striking incongruities were observed. That is at least six chromosomes could not be matched. Especially one chromosome was completely different from all possible partners, it had one very short and one very long arm ("hook-chromosome", ratio of arm length about 5/1, Fig. 15a, b).

2. Chromatin-Diminution

a) The Diminution Divisions. Cytological details of the diminution division, especially the behavior of the eliminated chromatin material during mitosis, have already been described for *C. divulsus* (S. Beermann, 1959). Diminution in *C. furcifer* basically follows the same pattern, but the species differ as to the time and duration of the diminution process. In *C. divulsus* elimination is completed in one step and is confined to the 5th cleavage division, which is preceded by a 3–4 h interphase. In *C. furcifer*, in contrast, diminution begins in the 6th cleavage division (preceded by an interphase of only 60 min), and

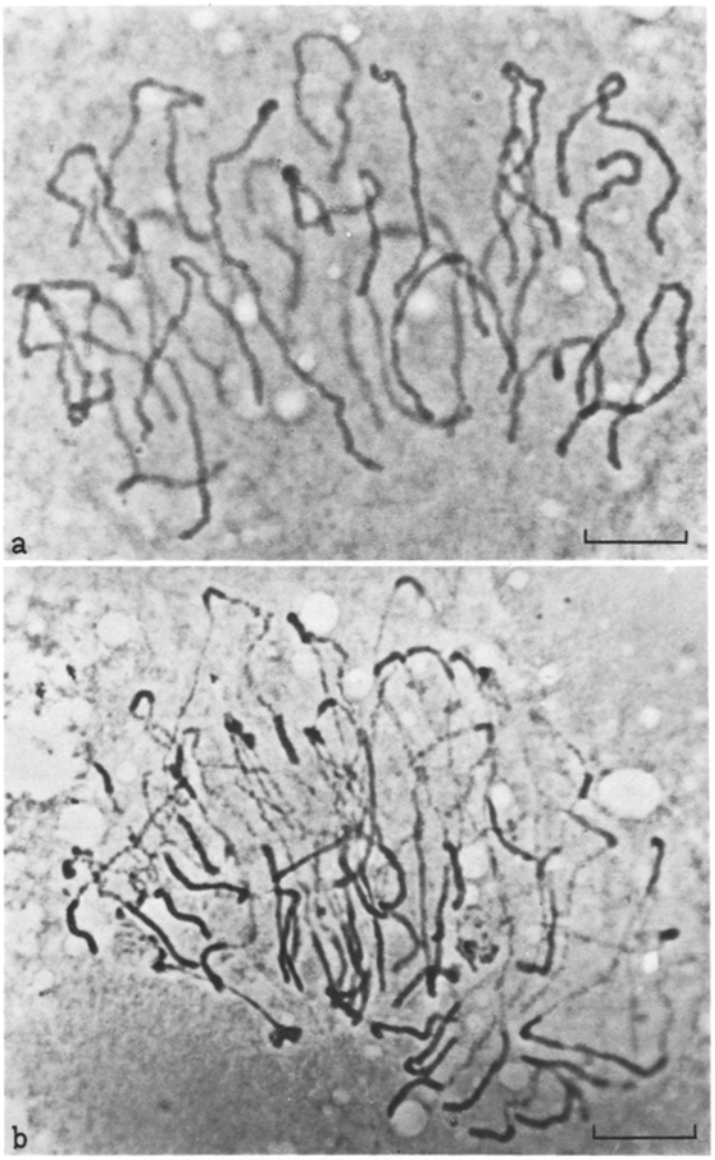

Fig. 12a and b. Differentiation of H-segments in *C. furcifer*: **a** Prophase of 3rd cleavage division, showing complete isopycnosis. **b** Prophase of 5th cleavage division, showing distinct heteropycnosis of terminal and kinetochoric H-segments

is not completed until after the 7th cleavage. Thus chromatin diminution in *C. furcifer* occurs in two steps. In the interphase before the 6th cleavage, the characteristic telophase distribution of heterochromatin is still maintained. One half of the nucleus contains large blocks of heterochromatin, which represent the terminal H-segments, whereas the other half of the nucleus displays a number

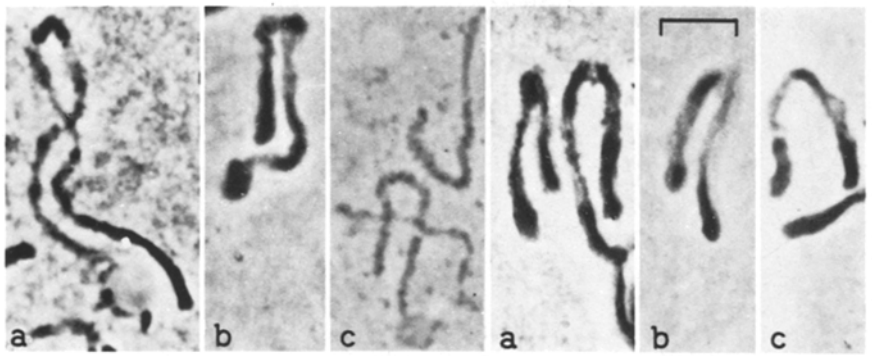

Fig. 13a–c. *C. furcifer*, nucleolar chromosome in mitosis: **a** Prophase 5th cleavage showing residual nucleolus at one end. **b** Pro-metaphase 5th cleavage, nucleolar H-segment condensed. **c** Prophase 7th cleavage without H-segments. Diminution already completed

Fig. 14a and b. *C. furcifer*, chromosomes from a pro-metaphase of 5th cleavage: **a** H-segment including kinetochore. **b, c** H-segment located to one side of the kinetochore. Bar equals 5 μm

of small chromocenters derived from the kinetochoric H-segments (S. Beermann, 1966: Fig. 1c). In order to follow up more closely the course of diminution, slides from the same batch of eggs were made at intervals of 10 min. The observations indicate that the H-segments are still replicated before the onset of diminution, i.e., early in the first 30 min of the interphase both terminal and kinetochoric chromocenters as a rule show a chromatid split. Cytophotometric data confirm this conclusion (S. Beermann, 1966). Only towards the end of the interphase the heterochromatin becomes less compact. In the early prophase, as soon as the individual chromosomes emerge as thin tangles of thread, the heterochromatin is no longer distributed in the typical manner. There are no distinct heterochromatic segments in the chromosomes, but the nucleoplasm contains a large number of chromatin particles (Fig. 16a). During the mitotic division the diminuted chromatin is eliminated from the spindle in the manner previously described for *C. divulses*. In the metaphase the chromatin particles are distributed throughout the entire spindle, but as soon as the anaphase movements are initiated they migrate into the equatorial plane and fuse together to form lumps of chromatin. These stretch out parallel to the axis of the spindle at telophase, but are not included in the telophase nuclei (Fig. 16a–d).

That portions of the heterochromatic segments have not yet been removed in the 6th division can be deduced from observations on the telophase and interphase nuclei (Figs. 16d, 19a). They contain small patches of condensed chromatin which may be considered as remnants of H-segments. The remaining heterochromatin is completely removed from the chromosomes in the next (7th) division in a manner completely analogous to the first diminution step (cf. S. Beermann, 1966).

b) Alteration of Chromosome Morphology as a Result of Diminution. The study of pre-diminution mitosis has revealed striking differences in lengths and arm ratios between paternal and maternal chromosomes. As follows from the dip-

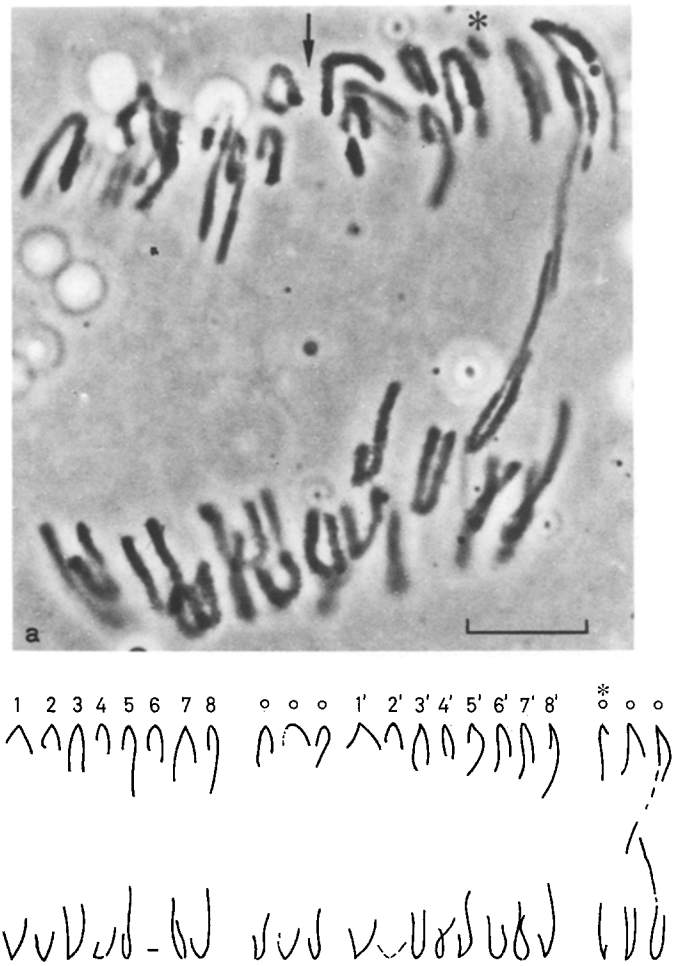

Fig. 15a and b. *C. furcifer*, anaphase 4th cleavage with gonomery as indicated by arrow: **a** One photograph from a series of three in different focal planes. **b** Semi-schematic diagram of the chromosomes, based on the series of photos. As far as possible the chromosomes are arranged into matching pairs; see numbers above chromosomes. For 3 of the chromosomes (o) no matching partner can be found. * Hook-chromosome

lotene analysis of heteromorphic bivalents these differences are entirely due to disparities in the H-segments and should therefore disappear as a consequence of diminution.

The chromosomes in the 7th cleavage are considerably shorter than those in the previous divisions. In 10 analyzed meta- and anaphases from different individuals, the 22 chromosomes could be arranged into 5 pairs of matched chromosomes with equal, and 6 pairs with unequal arms (Fig. 33b). A pair of hook-chromosomes can always be distinguished among the latter type (Fig. 17). No more than one hook-chromosome was observed before the diminution. The short, button-like arm of the other partner must, therefore, previously

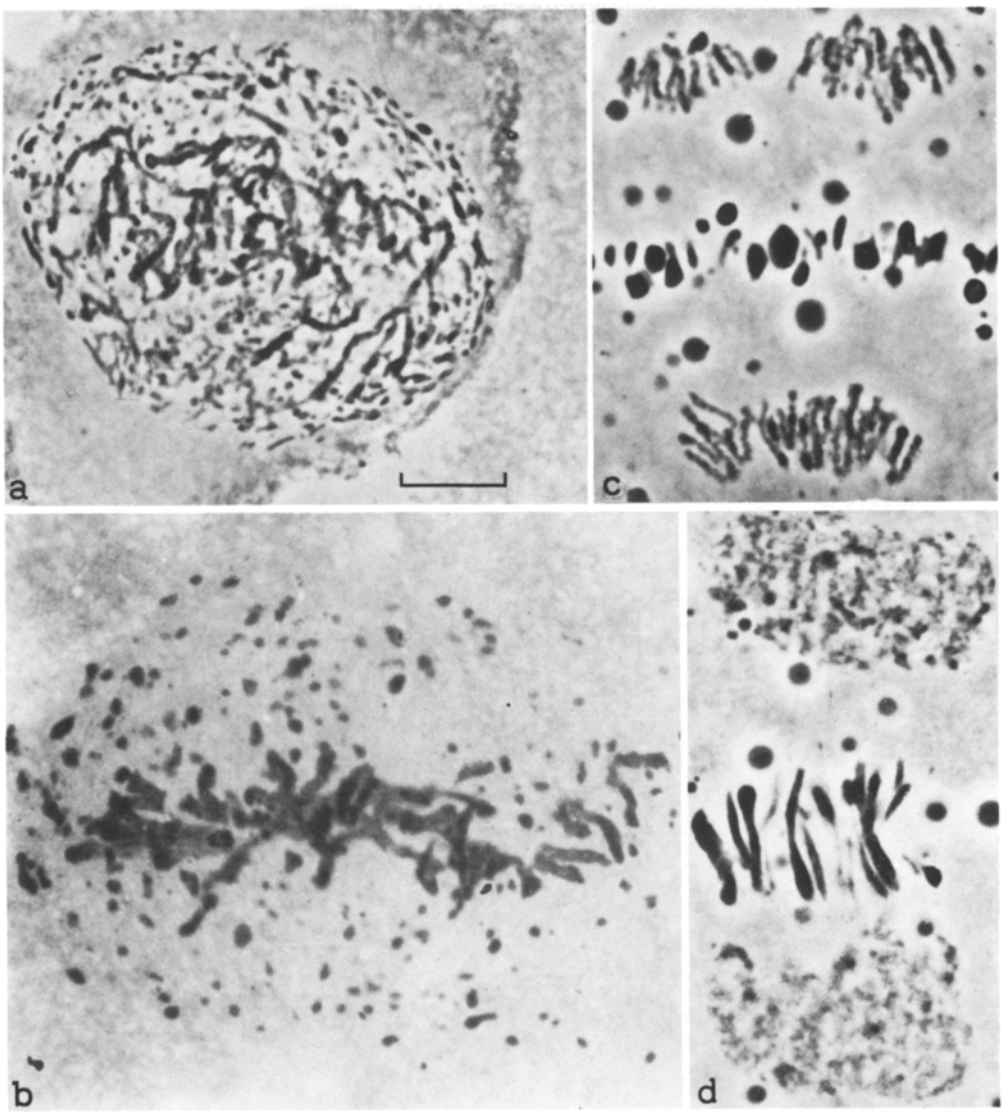

Fig. 16a–d. *C. furcifer*, chromatin diminution in the 6th cleavage: **a** Prophase, nucleoplasm filled with small chromatin particles. **b** Metaphase, chromatin particles distributed throughout the spindle. **c** Late anaphase, fused chromatin particles, located in the equatorial plane. **d** Telophase, eliminated chromatin remains outside of the newly formed nuclei

have been considerably longer. Evidently its reduction in size is due to the loss of a terminal H-segment. Among the other chromosomes only the nucleolar chromosome can be identified. In oogenesis and in the prophase of the 5th cleavage, it is characterized in addition to the nucleolus by two interstitial H-segments on the nucleolar arm. After diminution it still shows the terminal nucleolar organizer, but the H-segments are missing (Figs. 14 and 18). While

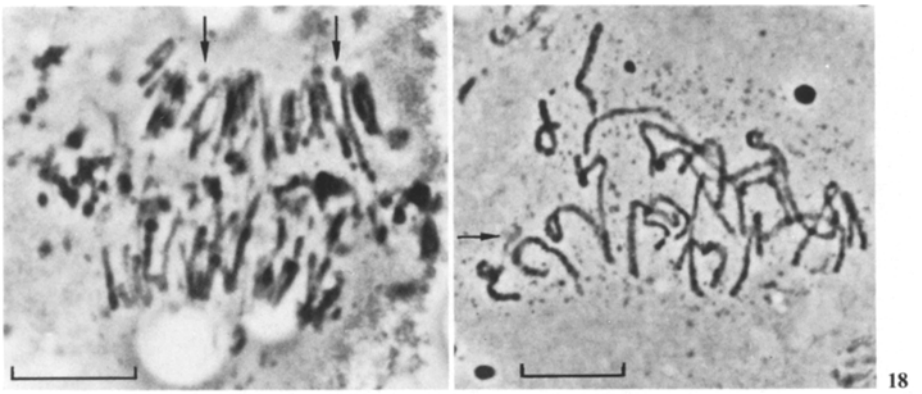

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Fig. 17. *C. furcifer*, diminution anaphase showing 2 hook chromosomes (arrow)

Fig. 18. *C. furcifer*, prophase of the 2nd diminution (7th cleavage). Few, very fine chromatin particles in the nucleus. Chromosomes without H-segments. One nucleolar chromosome with terminal nucleolus organizer (arrow)

it can be readily seen that the terminal H-segments in *C. furcifer* have been removed, it is more difficult to demonstrate this for the small blocks of heterochromatin at the kinetochores. The only morphological indication of their loss is the absence of chromocenters in interphase nuclei after the second diminution. That diminution really has taken place here can be derived from photometric data (S. Beermann, 1966), which will briefly be reviewed here.

c) Changes in DNA-Content. Cytophotometric measurements (see Materials and Methods) led to the following results: 1. Before the diminution there is about three times as much DNA in telophases as afterwards [in the population *C. furcifer* (BU) 2.2 times as much, compare Table 6 of this paper]. Therefore, more than half of the total DNA from the germ line genome is eliminated in the somatic cell nuclei of *C. furcifer*. 2. The DNA in the cell nuclei prior to the 6th cleavage division is completely replicated, as seen by comparing total anaphases of the 5th cleavage division with late interphases and metaphases of the 6th cleavage. 3. About 80% of the total heterochromatin is eliminated in the first diminution process; the remaining 20% are equally distributed between both daughter-telophases, and replicate once more prior to the second diminution.

With the aid of cytophotometry it was also possible to check the fate of the H-segments in the kinetochore regions. In late interphase nuclei, prior to diminution, the chromocenters representing the kinetochoric H-segments are spatially separated from the terminal ones (cf. Fig. 19a). The DNA content of those nuclear halves containing the kinetochores together with most of the euchromatin was compared to the 4C-value of late embryonic somatic mitoses. It was found that the DNA values from half-nuclei with non-diminuted kinetochores, were significantly higher than the 4C values from late embryonic mitoses. The results indicate that some of the measured DNA amounting to about 10 percent of the total germ line genome is lost during the 6th and 7th cleavage

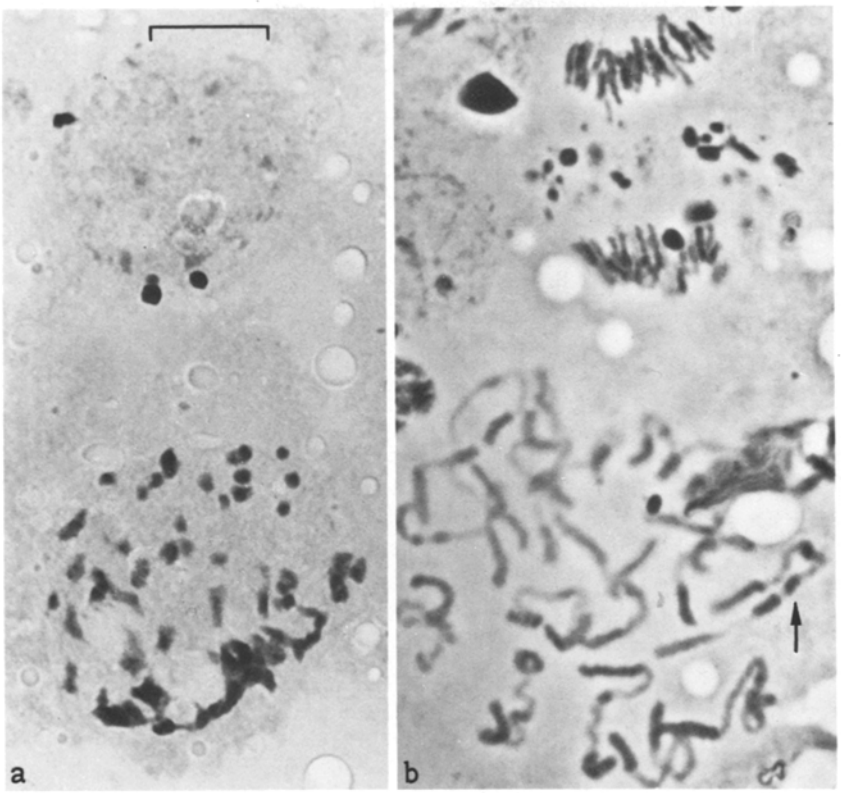

Fig. 19a and b. *C. furcifer*, 7th cleavage: **a** Interphase: primordial germ cell with chromocenters formed by the H-segments. Above is a palely stained somatic nucleus after the 1st diminution, with 3 lumps of eliminated chromatin outside of the nucleus. **b** Prophase of the primordial germ cell showing differentiation of H-segments. Nucleolar chromosome indicated by arrow. Above, a somatic cell in anaphase of the 2nd diminution

divisions, and it seems highly probable that this is the DNA of the H-segments in the kinetochore region.

III. Structure of Chromosomes and Chromatin Diminution in C. strenuus

From earlier observations it was known that chromatin is eliminated in one of the first five cleavage divisions in *C. strenuus* as in *C. divulsus* and *C. furcifer* (S. Beermann, 1959, Fig. 8). However, the cytology of this species was not immediately investigated because its chromosomes did not have well-defined eu- and heterochromatic segments, and because its early cleavage mitoses, especially the diminution division, seemed difficult to analyze. However, as will be shown, the chromosomes of this species proved especially valuable in elucidating the nature of chromatin diminution.

1. Distribution of Heterochromatin and Polymorphism of H-Segments in the Germ Line

a) Oogenesis in the Population Seehof (SE) and Hohenentringen (HO). In contrast to *C. divulsus* and *C. furcifer*, where the chromosomes in the germ line consist of well-defined euchromatic and heterochromatic segments, this is not the case in the investigated strains of *C. strenuus*. During its oogenesis heterochromatin appears to be distributed more or less randomly along the entire length of the chromosome. In the earliest oocytes, the presence of extra chromatin in the nuclei is obvious from their intense staining as compared with the surrounding somatic nuclei (Fig. 20a). However, the chromatin is not fused into lumps to such an extent as in the corresponding stages of *C. furcifer* (Fig. 5a). At the beginning of pachytene the bivalents appear as heavily but homogeneously stained threads, each of which has a distinct longitudinal split (Fig. 20a). With the onset of early diplotene in growing oocytes the chromosomes become longer and paler and in some segments poorly defined (Fig. 20b). They remain distance-paired and now display a loosened, lampbrush-like structure. Unlike in *C. furcifer* and *C. divulsus* this structure is here maintained even during later oocyte stages when both the nucleus and the chromosomes undergo contraction. In the loosened diplotene bivalents it is difficult to identify and localize heterochromatin. However, differences in chromatin content between different bivalents and between individual homologues are easily observed (Fig. 20c–e). In some bivalents both homologues carry equally large quantitites, in others equally small amounts of chromatin, and in a majority of bivalents they are clearly unequal, i.e. heterozygous, in this respect. In the investigated populations (SE) and (HO) no more than 3 of the 11 bivalents were really equal, and 8 were more or less unequal. These disparities can still be observed in early diakinesis, but after the bivalents have contracted into pairs of short, thick rods, the differences become indistinct (Fig. 22a). An interesting feature of diakinesis is the wide separation between homologues in each bivalent. Sometimes the gap is larger than the width of the chromosome. The bivalents are still arranged in this fashion when they form the metaphase plate of the 1st meiotic division in which they remain arrested until oviposition (Fig. 24a).

The observed variations on the amount and distribution of chromatin together with the fact that chromatin diminution is known to occur also in *C. strenuus*, strongly suggest that we are dealing with a case of interstitially located germ line limited heterochromatin. Proof for this interpretation comes from the study of a population of *C. strenuus* with an extreme type of dimorphism for the germ line heterochromatin. The detailed investigation was confined to this population.

b) Oogenesis in the Population R3. In females of the population (R3), each of the 11 bivalents typically consists of one large homologue with a high chromatin content and a small one with a low chromatin content. Only occasionally females are found homozygous for the chromosome type with a large amount of chromatin. Individuals homozygous for the small type of chromosome, or with mixed karyotypes, were never found.

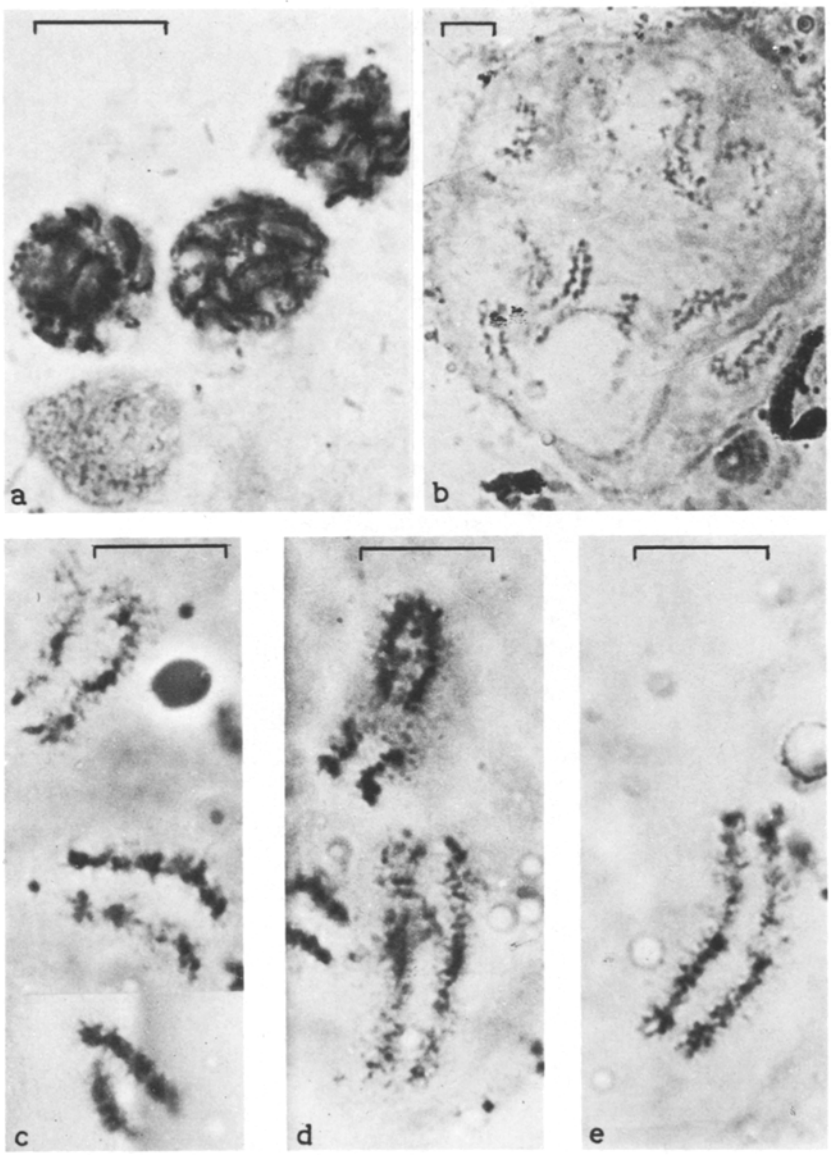

Fig. 20a–e. *C. strenuus* (Seehof, Hohenentringen), oogenesis: **a** Three oocyte nuclei in late pachytene, and below them a somatic nucleus. **b** Oocyte in the growth phase with diplotene bivalents. **c** Three distance-paired unequal bivalents in diplotene. **d, e** Three bivalents with equal distribution of heterochromatin in the homologues

The pachytene bivalents in females of *C. strenuus* (R3) do not look different from those of *C. strenuus* (SE). Striking disparities between homologues first become apparent towards the beginning of diplotene when the oocyte starts growing (Fig. 21 a). Separated individual bivalents show one homologue intensively stained and packed with chromatin particles of various sizes which are

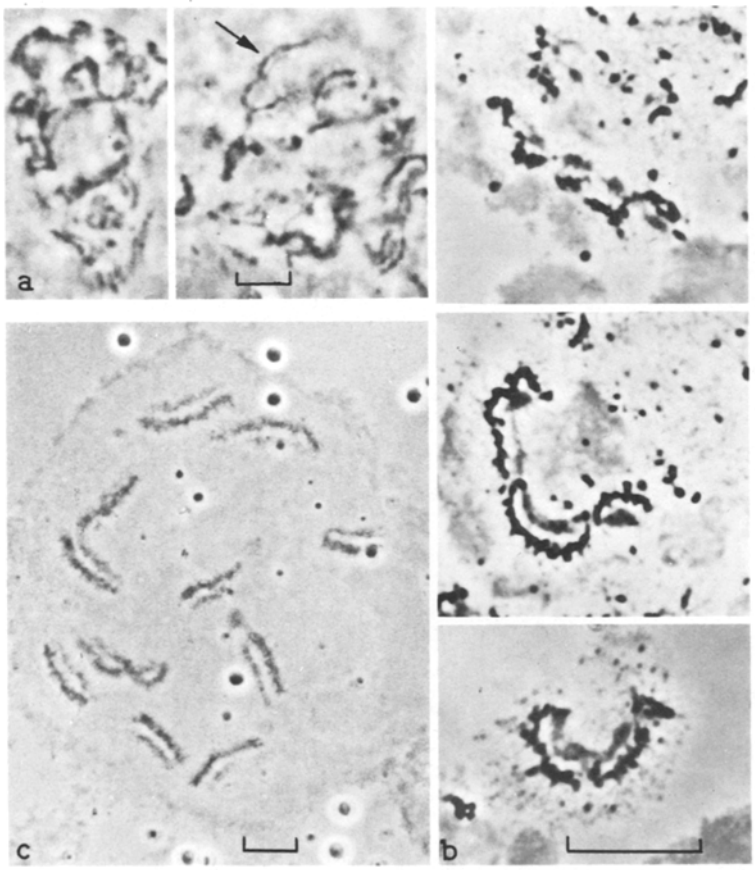

Fig. 21 a–c. *C. strenuus* (R3), oogenesis: **a** Two nuclei in early diplotene showing different chromatin content of the paired homologues (arrrow). **b** Three early diplotene bivalents at higher magnification. Well defined lampbrush-structure in the lower bivalent. **c** Oocyte nucleus with 11 bivalents in late diplotene. All bivalents unequal

missing in the weakly stained partner, indicating that it is primarily euchromatic (Fig. 21 b). Long euchromatic segments are never found in the heterochromatin-rich partner. In some bivalents their lampbrush-structure is rather conspicuous, and it appears that the loops protrude further from the more massive partner than they do from the thinner one while both are of the same length (Fig. 21 b, below).

During the following stages of oocyte growth the structure of the bivalents temporarily becomes less distinct, but in late diplotene the extreme differences in the sizes and staining intensities of both partners are once again apparent (Fig. 21 c). One partner is always much paler and thinner than the other one, but both remain distance-paired along their entire lengths and are therefore of the same length up until diakinesis, sometimes even metaphase I (Fig. 23). As a rule, however, in metaphase I the pale partner does become shorter so that each of the 11 bivalents comes to consist of a thicker and longer homologue

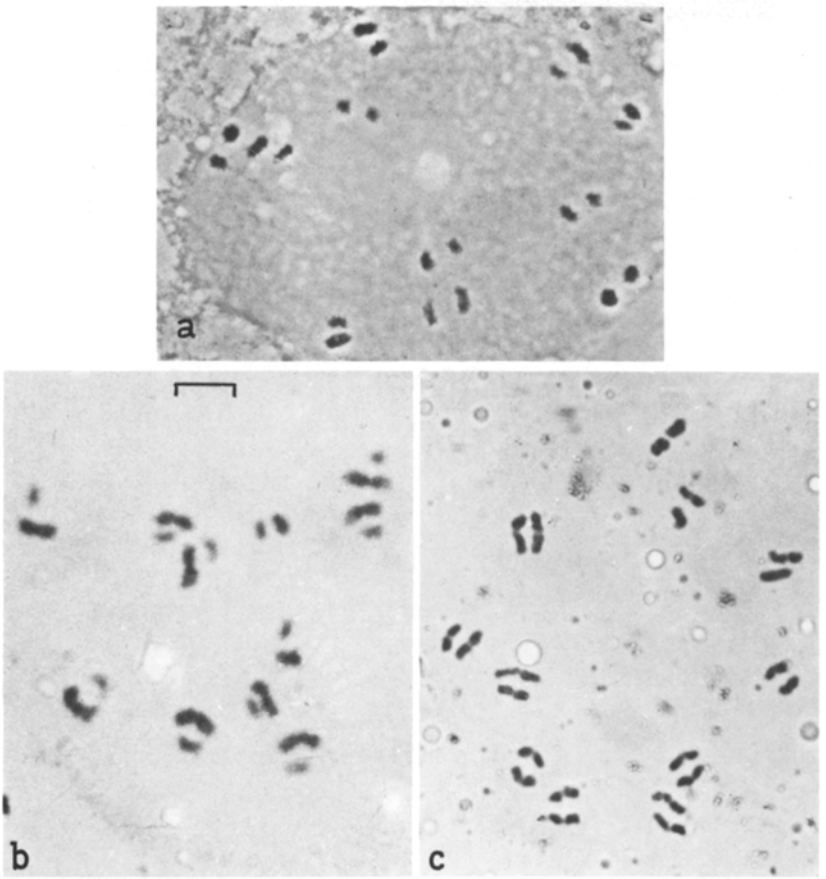

Fig. 22a–c. *C. strenuus*, oocyte nuclei with distance-paired bivalents in diakinesis: **a** Equal and unequal bivalents, female from the population Seehof. **b** Eleven unequal bivalents, female from the population Reusten (R3). **c** Eleven equal bivalents formed by the large chromosome type, female from the population Reusten (R3)

and a thinner and somewhat shorter one (Fig. 24b). Occasionally the contraction can occur already at diakinesis (Fig. 22b).

In metaphase I, the 11 bivalents of heterozygous (R3)-oocytes regularly arrange themselves in such a fashion that all of the large dyads face towards one spindle pole, and all of the small ones towards the other (Fig. 24b). This represents an extreme case of coordinated orientation and segregation. As in all Copepods, there is no interkinesis, and meiosis II immediately follows anaphase I. In the second meiotic division, therefore, one of the daughter-spindles contains only large chromosomes, and the other one only small ones. Thus, depending upon the original orientation of the bivalents the female pronucleus either carries only large or only small chromosomes. As in the other species, chiasmata have never been observed. If crossing-over did occur, it should be easy to discover in such an extreme case of dimorphism. However, no cross-overs between large and small chromatids have ever been noted.

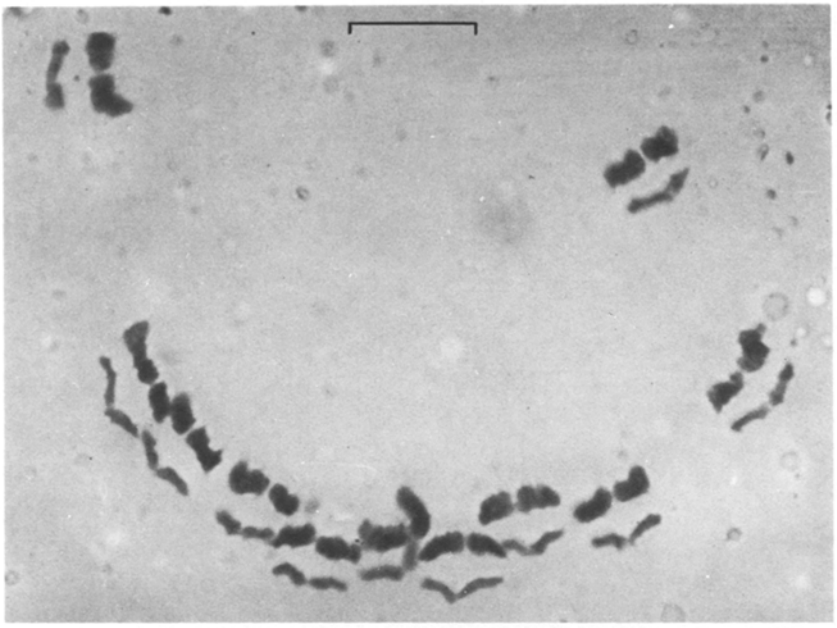

Fig. 23. *C. strenuus* (R3), oocyte nucleus in pro-metaphase I. Coordinated orientation of large and small homologues in all bivalents. Note equal length of small and large partners

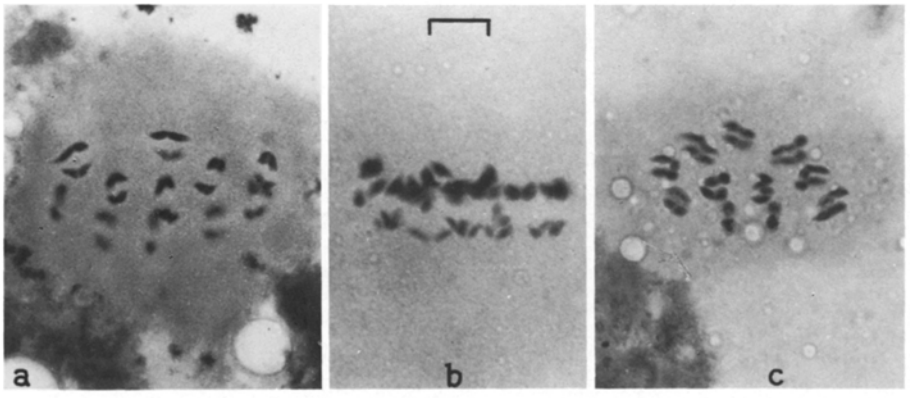

Fig. 24a–c. *C. strenuus,* oocytes in metaphase I: **a** Population Seehof: equal and unequal bivalents. **b** Population (R3): dimorphy and coordinated orientation in all 11 bivalents. **c** Population (R3): 11 equal bivalents of the large chromosome type

As mentioned above, some of the females in the population (R3) of *C. strenuus* are homozygous for the heterochromatin-rich set of chromosomes (Figs. 22c, 24c). In five test counts made between winter 1974 and spring 1976, the ratios of homozygous to heterozygous females were 8/29, 1/15, 0/20, 1/21 and 0/32, which give a total of 10/117, i.e., a 7.8% frequency of homozygotes.

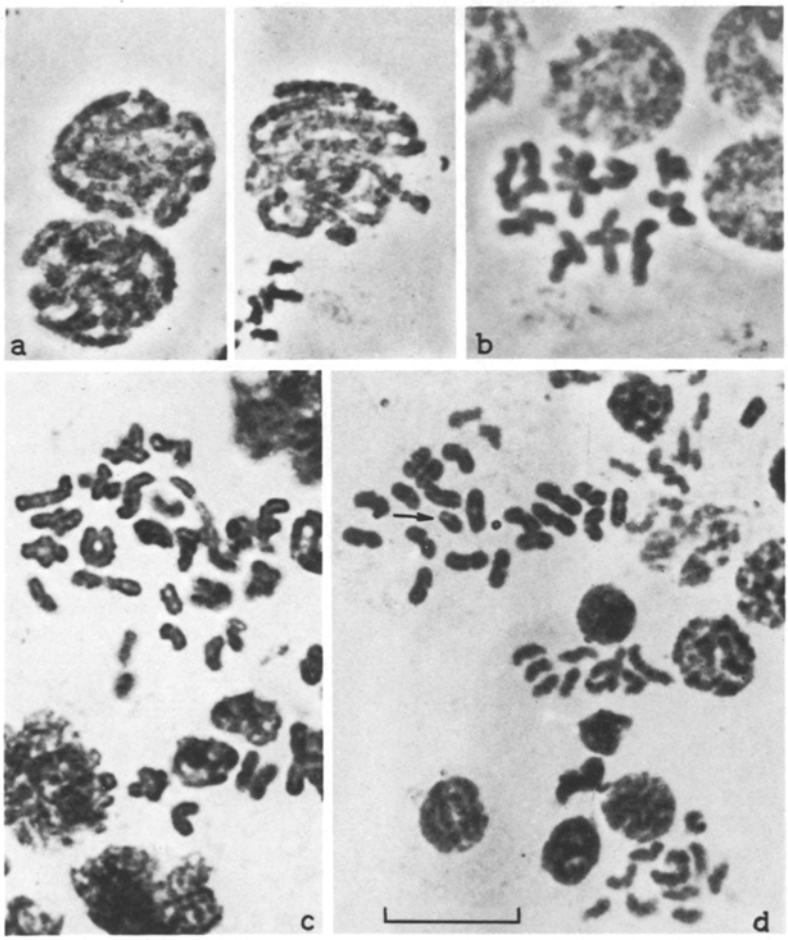

Fig. 25a–d. *C. strenuus* (R3), spermatogenesis: **a** Pachytene. **b** Metaphase I: symmetric bivalents with chiasmata. **c** Chiasmatic bivalents and some univalents from squashed metaphases. **d** Upper half: 10 equal pairs of dyads and one small dyad whose partner is missing (arrow), presumably anaphase I. Lower half: late anaphase II with 11 chromosomes at either pole

c) Spermatogenesis. In order to understand the striking heterochromatin dimorphism in the germ line of the R3 females, it is necessary to study the cytological situation in males. Spermatogenesis in *C. strenuus* as in other Copepods is more difficult to analyze than oogenesis. The only well defined stage of meiotic prophase is pachytene. Pachytene bivalents appear homogeneously stained and of even thickness along their length. They show a clear pairing split (Fig. 25a). A differentiation into eu- and heterochromatin cannot be recognized. Only the staining intensity, which is much stronger than that in somatic nuclei suggests the presence of large amounts of extra chromatin. Following pachytene both the nucleus and the chromosomes contract, typical diplotene and diakinesis stages have not been identified. At metaphase I the chromosomes still are closely

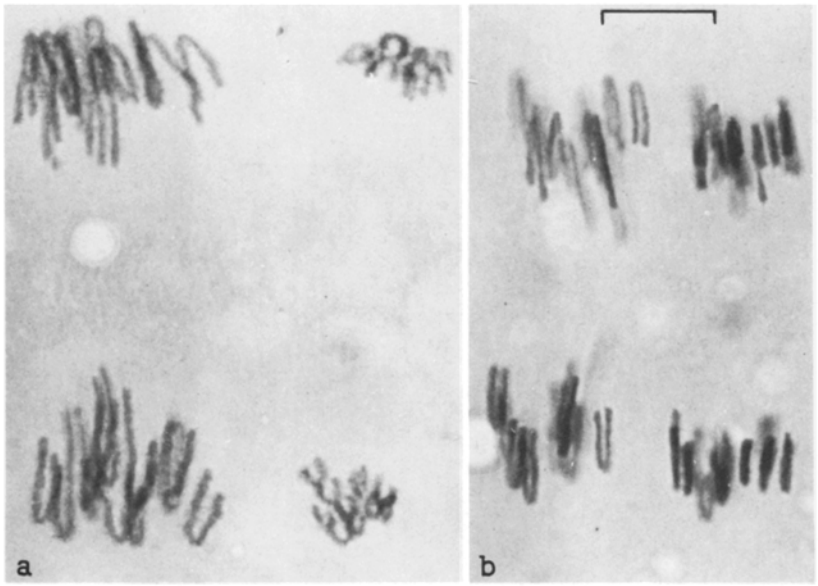

Fig. 26a and b. *C. strenuus* (R3), late anaphase of 2nd cleavage mitosis with clear separation of paternal and maternal chromosomes (gonomery): **a** Heterozygous egg: large paternal chromosomes to the left, small maternal chromosomes to the right. **b** Homozygous egg: large paternal chromosomes to the left, and large maternal chromosomes to the right

packed, and their orientation in the equatorial plane can hardly be discerned. Nevertheless it was possible to analyze a few metaphases I with all 11 bivalents. Some of these had clearly visible interstitial chiasmata (Fig. 25b, c), and the bivalents were always symmetrical. After the dyads have been separated in anaphase I the chromatid split and the kinetochoric gap are well-defined (Fig. 25d). Except for one very small chromosome found in some males the chromosomes are all of about the same size (Fig. 25d). The same is true for the second meiotic division (Fig. 25d, below).

In at least 30 investigated males a structural heterozygosity such as that observed in females was never found. The homologues in each bivalent were always identical. In addition, the sperm nuclei were all of the same size, a finding which would not be expected in the case of an extreme heterozygosity for heterochromatin content (Fig. 34c). These observations indicate that males of the population (R3) are homozygous for the heterochromatin-rich set of chromosomes.

d) Early Cleavage Divisions. Until the 4th cleavage division of *C. strenuus* (R3) all chromosomes are evenly stained along their lengths. The disparities in chromatin content as found in oogenesis are expressed only as variations in length (Fig. 26a). As a consequence of the directed reduction described above, only two types of female pronuclei are formed in heterozygous females, one with a set of heterochromatin-rich chromosomes and the other with a low heterochromatin set. The data from spermatogenesis, on the other hand, show that the

Table 4. Numbers of embryos of *C. strenuus* (R3) homozygous, and heterozygous, for the heterochromatin rich genome

Eggs deposited at	Slide number	Cleavage division	Mitotic stage	Embryos		
				homo-zygous	hetero-zygous	doubt-ful
18–20° C	(R3)/212	1st	meta-anaphase	8	8	–
	a_1/a_2	2nd	meta-anaphase	14	19	–
	20/2a	3rd	prophase-metaphase	9	–	3
		3rd	anaphase	–	6	–
	20/2b[a]	3rd	ana-telophase	22	1	1
		3rd	telo-interphase	–	–	24
	Sum total			53	34	28
4° C	4/1b	2nd	prophase-metaphase	5	11	8[b]
		2nd	anaphase	2	18	–
	4/2b	2nd	prophase-metaphase	16	20	14
		2nd	anaphase	–	4	–
	4/3b	3rd	anaphase	7	17	8[c]
	4/6b	3rd	anaphase	6	18	10[c]
	Sum total			36	88	40

[a] 10 min later than 20/2a; [b] prophase; [c] late telophase

males always contribute a heterochromatin-rich set. Therefore, only two types of zygotes should result, i.e., homozygous heterochromatin-rich and heterozygous ones. Mitotic division figures from the first through 3rd cleavage divisions confirm this. About half of the eggs has one set of short chromosomes and one set of long ones, whereas the other half contains only long chromosomes (Fig. 26). In scoring the numbers of homozygous and heterozygous embryos, it was important to count all eggs from a given batch, including those which were not clearly analyzable. This is necessary since heterozygotes develop faster than the homozygotes. As a result one of the two classes could preferentially be in a not analyzable stage, such as prophase and late telophase. Table 4 shows an instructive example. In the slide 20/2b, the 22 homozygous embryos are in late anaphase, whereas 25 not clearly analyzable embryos are in late telophase or early interphase.

Most of the latter must be heterozygous. As can be seen from the data in the table, about half of the eggs laid at room temperature (18–20° C) have the homozygous heterochromatin-rich genotype, and about half are heterozygous (53 homozygotes: 62 heterozygotes, or presumed heterozygotes). When the animals are bred and the eggs laid at lower temperatures (4° C), in contrast, clear indications exist that the ratio is displaced in favor of the heterozygotes. At least in the egg batches 4/1 b, 4/3 b and 4/6 b, the proportion of homozygotes is much less than 50%. In addition these data show that zygotes with two

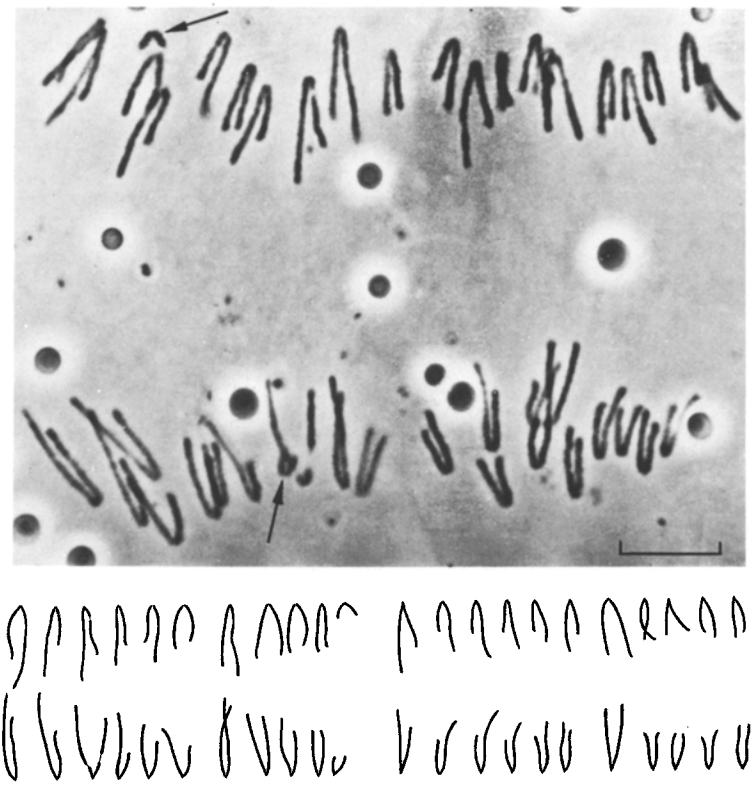

Fig. 27. *C. strenuus* (R3), 2nd cleavage mitosis from a homozygous egg. In the diagram the chromosomes of both sets are arranged according to size and arm ratio. The paternal set (left) in this case has one small chromosome (arrow)

small, low-heterochromatin sets of chromosomes never occur. The results confirm the conclusion that only sperms with heterochromatin-rich chromosomes are formed in R3 males.

The small chromosomes in the heterozygous nuclei must, therefore, be of maternal origin. The large ones can be derived from either parent. Since the set of small chromosomes is exclusively inherited by females, that set must contain a factor which determines femaleness. This constitutes a proof for female heterogamety in *C. strenuus* (comp. W. Beermann, 1954; Rüsch, 1960, and Discussion).

When individual chromosomes of the large set are arranged on the basis of their sizes and the relative lengths of their arms, 6 of them appear with unequal arms, and 5 are equal armed ones (Fig.27). The paternal set of large chromosomes in some cases contained a very small chromosome (Fig. 27). Conversely heterozygous females sometimes have one chromosome larger than the others in their set of small chromosomes. In homozygous embryos, the chromosomes of maternal origin are slightly more condensed, and therefore slightly shorter than chromosomes of paternal origin (Figs. 26 and 27). In the set of

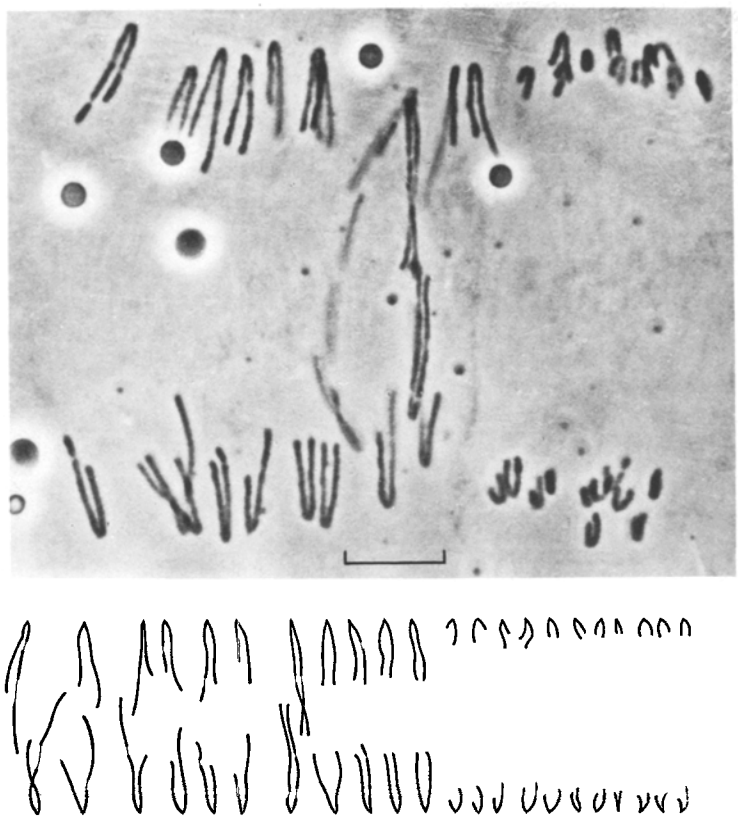

Fig. 28. *C. strenuus* (R3), 2nd cleavage mitosis from a heterozygous egg. In the diagram the chromosomes of both sets are arranged according to size and arm ratio

Table 5. Sum of chromosome lengths in the large and in the small set from 1st or 2nd cleavage anaphases in *C. strenuus* (R3)

Slide number	Total length in arbitrary units		Ratio large/small
	large set	small set	
22/23	392,5	105.5	3.7:1
20/21	366.5	113.5	3.2:1
16/17	314.5	94.0	3.3:1

small chromosomes there were 8 equal armed and 3 unequal armed chromosomes (Fig. 28).

As seen in Figures 26a and 28, the difference in length between the small and the large chromosomes are quite considerable. If the lengths of the chromosomes of each set are summed up a ratio of approximately 3:1 between both sets results (Table 5). This difference is rather equally distributed among all of the chromosomes. Considering that in the early cleavage divisions the maternal set is somewhat more condensed than the paternal one, the length comparison

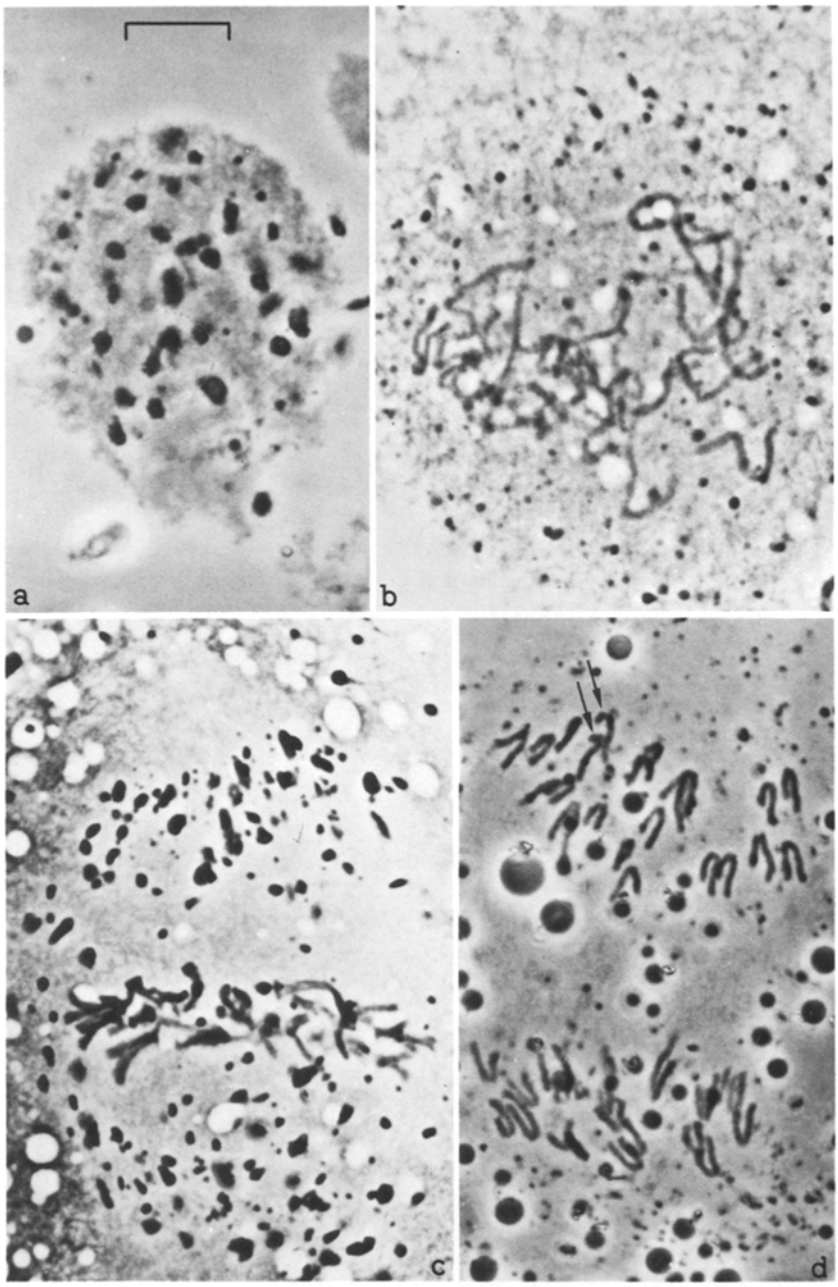

Fig. 29a–d. *C. strenuus* (R3), chromatin diminution in the 4th cleavage: **a** Diminuted chromatin in late interphase immediately preceding the elimination division ("dotted nucleus"). **b** Prophase with small chromatin particles. **c** Metaphase, chromatin particles at the spindle poles. **d** Anaphase, chromatin particles remaining at the spindle poles. Hook chromosomes shown by arrows. **a, c** size and number of chromatin particles indicate derivation from a homozygous embryo. **b, d** presumably from a heterozygous embryo

allows a first approximation of the amount of germ line limited chromatin in the chromatin-rich set, i.e., the set of large chromosomes must contain between 2 and 3 times as much DNA as the samll one. This is in accordance with DNA measurements (see page 333).

2. Chromatin Diminution

a) The Diminution Division. The process of diminution in *C. strenuus* differs from that in *C. divulsus* and *C. furcifer* in several respects. Diminution occurs already in the 4th cleavage, following an unusually long interphase of 7 to 8 h (Fig. 4 and Table 3). Unlike in *C. furcifer* and in *C. divulsus,* where the H-segments become visible before diminution, this is not the case in *C. strenuus.* Almost up to the end of the long interphase the nuclei maintain a swollen and weakly stained condition with the exception of the primordial germ cell (PGC) whose nucleus acquires a somewhat pycnotic appearance. Only 10 to 20 min before the beginning of the 4th division, numerous lumps of chromatin appear at the periphery of the nuclei (Fig. 29a). This indicates that chromatin has already been removed from the chromosomes at an earlier stage in the cell cycle than in both of the other species. In prophase the chromatin particles can be seen to be scattered throughout the nucleus while the chromosomes tend to aggregate in the center (Fig. 29b). At metaphase the eliminated chromatin material is accumulated at the spindle poles (Fig. 29c). However, in contrast to *C. divulsus* and *C. furcifer* it remains there throughout ana- and telophase (Figs 29d and 30). The amount of eliminated chromatin in all of the blastomeres from a given egg is identical, but varies in different embryos (comp. Fig. 29c and d). Those with a smaller amount of eliminated material are presumably heterozygous and those with a large amount homozygous for the heterochromatin-rich chromosomes.

b) Alterations of Chromosome Morphology as a Result of Diminution. According to the observations on oogenesis, the disparities in size between the small and the large chromosomes during the 1st to 3rd cleavage mitoses must be due to disparities in their content of germ line limited chromatin. The loss of the latter would be expected to have an effect on the relative and absolute dimensions of the chromosomes, just as in the other investigated species. In the present case, the heterochromatin-rich chromosomes should be altered to a much greater extent than the small, almost entirely euchromatic ones, and after the diminution all of the differences between both types of chromosome sets should disappear. Subsequent to the 4th cleavage, mitotic figures with one or two sets of large chromosomes are indeed no longer seen, with the exception of the primordial germ cells (Fig. 31).

In 4 completely analyzed anaphases from the 4th cleavage the somatic karyotype contained 5 metacentric and 6 submetacentric to subtelocentric pairs of chromosomes (Fig. 32). Among the latter was a pair of hook-shaped chromosomes with the arm length ratio of 1:5. In 24 analyzable meta- and anaphases from the 4th cleavage, two hook chromosomes were always found. Prior to

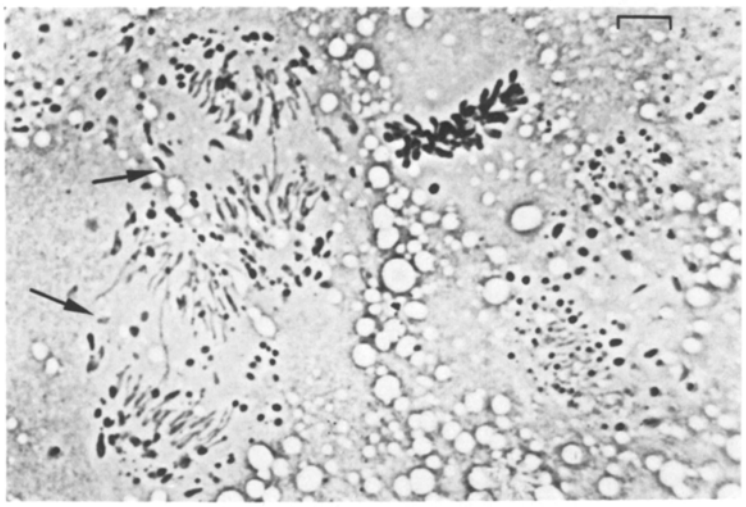

Fig. 30. *C. strenuus* (R3), 4th cleavage: Three diminution mitoses (2 anaphases (arrow), 1 telophase) and 1 primordial germ cell in metaphase in which chromatin is not being eliminated

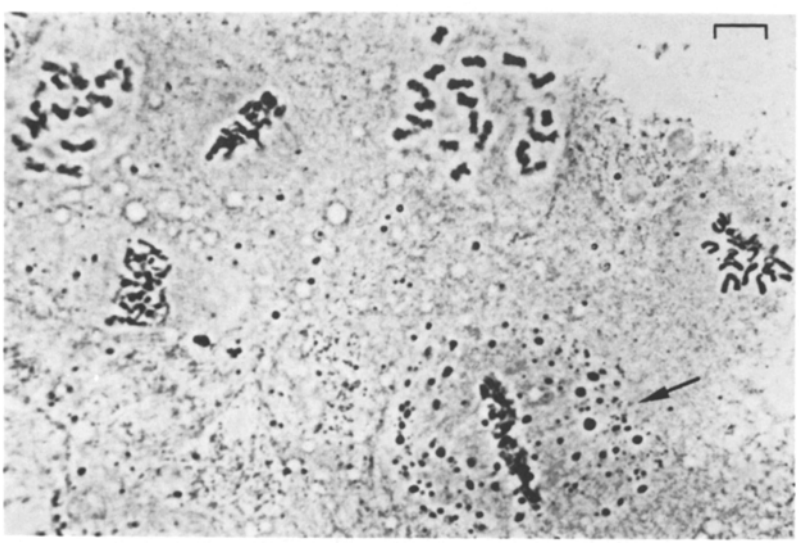

Fig. 31. *C. strenuus* (R3), 5th cleavage: 3 post-diminution metaphases, 1 metaphase still in diminution (primordial entoderm cell, arrow) and 2 primordial germs cells with condensed undiminuted chromosomes in interphase

the diminution, in contrast, none of the chromosomes of the large set had a chromosome arm ratio like this one (comp. Figs 27, 28). Even in the set of small chromosomes an element resembling the hook chromosome could not be identified with certainty. The inference that the set of small chromosomes also undergoes some alterations by the diminution is further strengthened by the fact that prior to diminution the small set has less unequal armed and more equal armed chromosomes (e.g., 3:8, or 4:7), than afterwards (6:5).

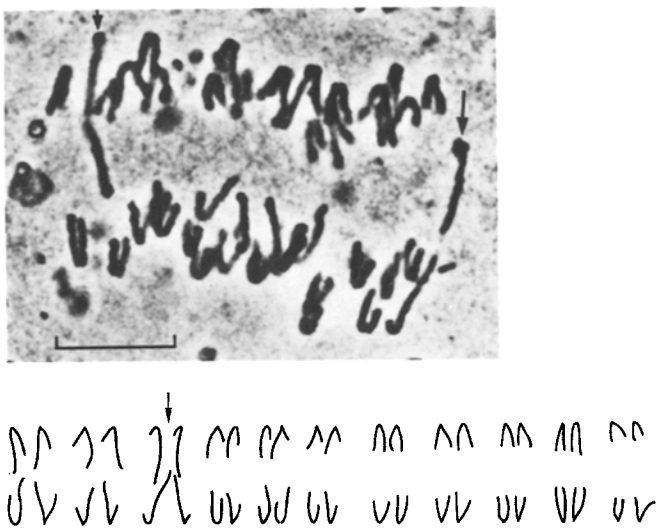

Fig. 32. *C. strenuus* (R3), anaphase 4th cleavage with diminuted chromosomes: In the diagram the chromosomes are arranged in matching pairs according to size and arm ratio. Hook chromosomes indicated by arrows

IV. Comparison of Chromosomes and DNA Content of the Genomes of C. divulsus, C. furcifer and C. strenuus

a) The Chromosomal Constitution After Diminution. In all three species the diminution completely eliminates the often quite considerable differences in size between homologous chromosomes. In other words, the chromosome polymorphism is confined to the heterochromatin of the germ line. Regardless of whether the heterochromatin occurs as unitary blocks on the ends of the chromosomes or at the kinetochores, or whether it is distributed as small segments along the entire chromosome, after diminution there are always 22 pairs of identical homologues in the diploid somatic nuclei.

Beyond this equality in chromosome number the somatic karyotypes of the three species also exhibit similarities in chromosome shape, first indicated by the presence of the "hook chromosome" in all three species. The somatic complements were drawn from photographs, ordered according to size and shape, and arranged in matching pairs. 3 anaphases from *C. divulsus* were compared to 3 late metaphases of *C. furcifer* and to 3 anaphases from *C. strenuus* (Fig. 33). Obviously the range of chromosome sizes in the karyotype of the three species is very similar. Also, in each of the species there are 5 equal armed and 6 unequal armed pairs of homologues. With the exception of the "hook chromosome", the identification of individual chromosomes within these groups is difficult. Only slight differences exist between the 5 equal armed ones. Among the 6 unequal ones, two are always large and have an arm length ratio of 2:1, one is hook shaped and 3 are medium sized and sometimes have arm length ratios approaching that of the hook chromosome. In *C. divulsus* one of the large, unequal armed chromosomes has a terminal, threadlike nucleo-

Fig. 33a–c. Somatic (post-diminution) karyotypes of **a** *C. divulsus*, **b** *C. furcifer* and **c** *C. strenuus*. Some chromosomes could not be identified (—). Hook chromosomes shown by arrow

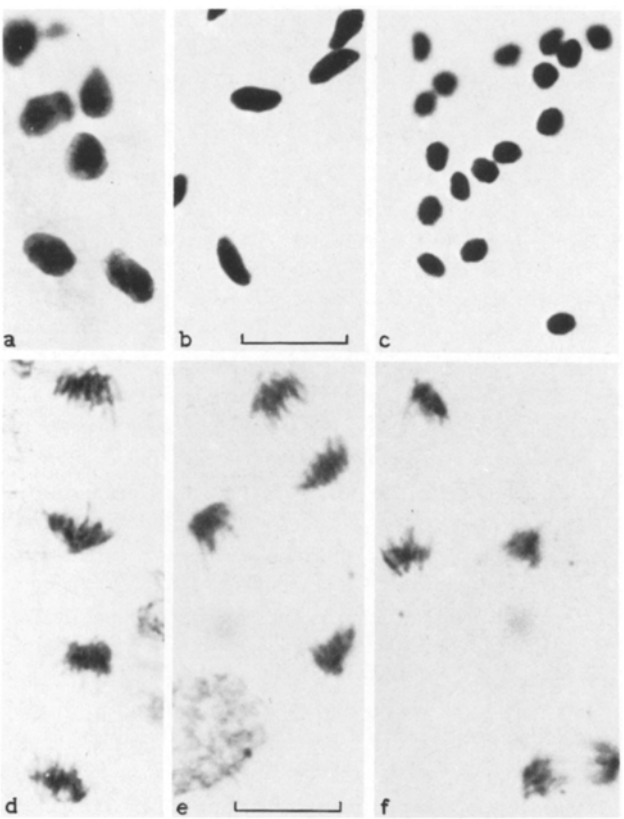

Fig. 34a–f. Sperm nuclei (**a, b, c**) and late embryonic anaphases **d**, **e**, **f** Feulgen stained for cytophotometry: **a**, **d** *C. divulsus* (SE), **b**, **e** *C. furcifer* (BU), **c**, **f** *C. strenuus* (R3)

Table 6. DNA content of sperm and embryonic anaphases from all three species determined by Feulgen cytophotometry

Species	Number of measure-ments	Mean absorbancy integral	Standard error of the mean	Estimated DNA content in 10^{-12} g at the 1C level
a) Sperm (=1C)				
C. strenuus (6 animals)	23	22.63	0.42	2.2
C. furcifer (4 animals)	22	30.88	0.44	2.9
C. divulsus (5 animals)	24	33.05	0.61	3.1
b) Somatic telophases after diminution (=2C)				
C. strenuus (4 egg batches)	26	19.77	0.30	0.9
C. furcifer (4 egg batches)	32	28.53	0.25	1.4
C. divulsus (5 egg batches)	33	37.36	0.39	1.8
Chick erythrocytes	29	26.46	0.30	

lar organizer on its short arm. In the nucleolar chromosomes of *C. furcifer* and *C. strenuus* the nucleolus is also terminally located.

b) The DNA Content of the Genomes. In spite of the conspicuous interspecific differences in the distribution of germ line heterochromatin, we have seen that the karyotypes of the somatic (i.e., diminuted) chromosomes of *C. divulsus, C. furcifer,* and *C. strenuus* are basically similar. In view of these observations, it is interesting to compare the DNA contents of the investigated genomes. In order to determine the somatic (diminuted) DNA content of the genome, telophase halves from late embryonic mitoses (=2C) were measured (Fig. 34). To determine the DNA content in the germ line sperm nuclei (=1C) (Fig. 34) were measured. Chick-erythrocytes were spread out on some slides as an internal standard for the determination of absolute amounts of DNA. According to Rasch et al. (1971), these nuclei contain 2.5×10^{-12} g of DNA.

The data show that there are significant interspecific differences in the DNA contents of the somatic genomes (Table 6b). The ratio of the values in *C. strenuus, C. furcifer* and *C. divulsus* respectively is 1:1.44:1.89. Therefore, in spite of the apparent similarities in karyotype, the sizes of the somatic genomes vary considerably.

The sperm nuclei of the three species also show clearly different DNA contents (Table 6a), but the disparities are not as great as those of the somatic genomes. As is expected, the values for the sperm are significantly higher than those for the soma. It must be noted, however, that in the case of *C. strenuus* (R3) the sperms are only representative for the large set of germ line chromosomes. If the sperm DNA value is compared to the 1C-value, calculated from measurements of somatic telophases, it can be seen that the germ line genome of *C. strenuus* has about 2.3 times as much DNA as the somatic genome. About 56% of the DNA is therefore germ line limited. The germ line genome of *C. furcifer* (BU) contains about 2.2 times as much DNA as the somatic

genome so that about 54% of the DNA is germ line limited. And finally, in *C. divulsus,* there is 1.8 times as much DNA in the germ line genome as in the soma, i.e., about 45% of the DNA is germ line limited. Expressed in absolute values, this means that the (large) set of germ line chromosomes in *C. strenuus* loses about 1.3×10^{-12} g of DNA through diminution, whereas that of *C. furcifer* loses about 1.5×10^{-12} g and that of *C.divulsus* loses about 1.3×10^{-12} g. Therefore the three species, although their somatic genomes are of appreciably different sizes, all have about the same amount of germ line limited DNA.

D. Discussion

I. Chromosomal Differentiation Between Soma and Germ Line

1. General Considerations

In most organims the chromosomes of the somatic cells are identical to those of the germ line. There are a few exceptions, however, in which the germ line carries supernumerary chromosomes (Cecidomyiidae, Sciaridae, Chironomidae) or supernumerary chromosomal segments (*Ascaris* and Copepods). All of these organisms belong to groups in which there is a strict separation between germ line and soma already in the earliest cleavage stages. The differential chromosome or chromatin distribution is always brought about by an elimination process early in development as a result of which the extra chromosomes or the extra chromosome segments are expelled from all nuclei of the presumptive soma, but not from those of the primordial germ cells. The latter therefore, carry in addition to the somatic genome chromosomes, or chromosomal segments, which are germ line limited. This material is heteropycnotic during most stages of development.

In *Cyclops*, as in *Ascaris*, the germ line limited chromatin exists as extra heterochromatic segments (H-segments). Relationships similar to those in *C. divulsus* are found in *Ascaris lumbricoides* with terminal H-segments on all of the chromosomes. However, the details of early development and the course of events leading to chromosomal differentiation are different in the two groups of organisms. While in *Ascaris* diminution first occurs already in the second cleavage at the division of the first somatic stem cell, and is repeated in the stem cells of the soma in the following cleavages, there is in *Cyclops* no apparent chromosomal differentiation up to the 4th cleavage at the earliest. Then all of the presumptive soma cells eliminate nearly simultaneously. Furthermore, again in contrast to *Ascaris*, the process of diminution as such can occupy two successive division steps for its completion, as found in *C. furcifer.*

It is interesting that species as closely related as *C. divulsus, C. furcifer* and *C. strenuus* differ to such an extent in their program of diminution and the timetable of early development. As shown in Results, the diminution occurs during the 4th cleavage in *C. strenuus,* during the 5th cleavage in *C. divulsus,* and during the 6th and 7th ones in *C. furcifer* (Figs. 2–4 and Tables 1–3). How-

ever, it has to be considered that a resting stage of 7 h precedes the 4th cleavage in *C. strenuus*, whereas in *C. divulsus* the corresponding phase before the 5th cleavage merely lasts 4 to 5 h, and in *C. furcifer* only a 1 h pause is interposed before the 6th cleavage (to which must be added the 2 h interphase prior to the 5th cleavage). Taking into account the uncertainty as to the actual beginning of diminution during the long interphase, especially in *C. strenuus*, the data are not in conflict with the view that the time from oviposition to diminution is just about the same in all of the three species. The same holds true for the time of the final differentiation of the primordial germ cells. It seems, therefore, that the different patterns of cleavage in the three species are really superimposed by an identical physiological time table, at least until gastrulation.

2. Controlling Factors of Diminution

From his experiments with *Ascaris*, Boveri (1910) concluded that there are cytoplasmatic factors which induce, and others which possibly inhibit, diminution. This view has recently been supported by the results of Moritz (1967). More detailed investigations of the mechanism controlling the elimination and its relation to soma-germ line differentiation were carried out by Geyer-Duszynska (1958, 1959, 1966) on the Cecidomyiid *Wachtiella*. She came to the conclusion that the entire cytoplasm of the egg contains factors which induce elimination. The elimination is prevented by a cytoplasmic component ("homogeneous substance"), intensively stained with haematoxylin, which is normally located only in the pole plasm.

Cytoplasmic components characterizing the early germ line have also been found in copepods in the form of the ectosomes. Amma (1911) showed that the ectosomes are correlated with the differentiation of the germ line in 16 investigated species of copepods. In most of these, however, chromatin diminution does not occur. It is possible that cytoplasmic inclusions such as ectosomes inhibit chromatin diminution in Copepod species with germ line limited chromatin, just as E-chromosome elimination is inhibited by a specific component in the pole plasm in Cecidomyiids. Although the necessary experimental evidence is not yet available, it is reasonable to assume that the mechanism of diminution in the somatic cells in *Cyclops* involves the synthesis and activation of a series of enzymes, processes which in Copepods probably are set in action only during the gastrulation. This phase of generalized gene activation seems to correspond with the long interphase preceding the diminution divisions. In order to prevent the activation of the diminution genes in the primordial germ cells it would suffice merely to suppress all of the somatic gene activities in these cells. It is possible that the ectosomes either contain an unspecific repressor with this function or induce its formation.

3. Biological Significance of Germ Line Limited Chromatin

In discussing the possible function of the extra chromatin in the germ line, the following two facts must be taken into consideration: 1. In those three

taxonoomic groups (Diptera, Copepoda, Nematoda), in which species possessing extra germ line chromatin have been found, these species constitute only minorities. It is therefore improbable that the elimination of extra chromatin serves as a basic mechanism controlling germ line differentiation. 2. The quantity of extra material varies considerably, especially from species to species, but also within species (comp. Bauer and W. Beermann, 1952). This point, taken together with the first one, seems to counterindicate the localization of ordinary gene functions, except for very unspecific ones, in the extra material. This is furthermore supported by the fact that the germ line limited chromatin segments of *Cyclops* and *Ascaris* appear as heterochromatic throughout almost all of the investigated stages of germ line development. The heterochromatic aspect of the extra H-segments suggests that they mainly contain satellite DNA with highly repetivive, simple sequences. Tobler et al. (1971), however, maintained that the germ line limited chromatin has larger amounts of non-repetitive DNS sequences in *Ascaris lumbricoides*. Moritz and Roth (1975), in contrast, present data to show that the germ line limited chromatin in this species contains primarily repetitive DNA which is missing in the soma.

From experiments involving ^{3}H-uridine incorporation in ovaries of Cecidomyiids, Kunz et al. (1970) conclude that the despiralized E-chromosomes synthesize RNA during oocyte growth. It must be taken into consideration, however, that the germ line chromosomes of the Cecidomyiids may have been derived from the somatic chromosomes by polyploidization. It is thus possible that utilizable residual information is activated in the germ line. Using density gradient centrifugation, Kunz and Eckhardt (1974) were not able to find any differences between germ line and somatic DNA in the gall midge *Heteropeza*.

II. Properties and Variability of Germ Line Heterochromatin

1. Cytological Characteristics

Heitz (1929) defined as heterochromatin regions of the chromosomes which remain condensed and intensively staining throughout interphase and prophase. These criteria apply to the germ line limited segments, especially in the stages of meiotic prophase, and also during the mitotic stages of the 5th and 6th cleavages in *C. furcifer* (and *C. divulsus* in the 5th cleavage). In *C. strenuus* the heterochromatic nature of the germ line limited material can only be inferred from the situation in oocytes during diplotene. In heterozygous (R3) females at this stage one of the partners in each bivalent is strongly laden with intensively stained, pycnotic material, while the pale one looks completely devoid of heterochromatin. Heterochromatin does not necessarily appear heteropycnotic in all stages of the life cycle of an organism. The three *Cyclops* species studied here furnish a remarkable example. Owing to complete isopycnosis of the H-segments during the first three or four cleavage divisions, the chromosomes appear entirely euchromatic along their length and are completely despiralized during interphase without forming chromocenters. Among the other established criteria for heterochromatin, i.e., late replication, C-banding and high content of repetitive

DNA, only the last would seem to be applicable here (see Moritz and Roth, 1975, for the case of *Ascaris*), the other two being always correlated with differential condensation.

It seems that the visible differentiation between hetero- and euchromatin, which first becomes apparent during gastrulation in *Cyclops,* requires the availability of specific chromosomal proteins which have either not yet been synthesized or are not yet active in freshly laid eggs. Possible candidates are the histones. In sea urchins, for instance, Ruderman and Gross (1974) found that a new, very lysine-rich, fraction of histones appears in early development, morula or early gastrula stages. Therefore, the "euchromatic" condition of the H-segments in early cleavage nuclei does not necessarily indicate transcriptional activity. On the contrary, it is more probable that the chromosomes at this time remain largely inactive as shown, for instance, by the fact that nucleoli are not formed. An absence of heteropycnosis during early development has, up to now, only been mentioned for *Drosophila* (comp. Cooper, 1959). However, the phenomenon may be more wide-spread. A certain analogy exists to the situation in mammals and mealy bugs, where entire chromosomes or sets of chromosomes also only become heteropycnotic after the first cleavage divisions (Garner and Lyon, 1971; Brown and Nur, 1964). In these cases, however, the heterochromatization is facultative (Brown, 1966).

In contrast to the situation found in early development the temporary lampbrush-like decondensation of H-segments during diplotene could indicate transcriptional activity. This point has not yet been checked.

2. Distribution of the Heterochromatin

The rules defined by Heitz (1929, 1932) for the localization of heterochromatin in plant and animal chromosomes are also valid for the germ line heterochromatin in the genomes of *Cyclops* species. Heterochromatic chromosomal segments are preferentially situated at the kinetochore region and at the ends of chromosomes, but occasionally distributed along the entire length of the chromosome. Heitz (1932) found that, when many or all of the chromosomes of a genome contain heterochromatic sites, these tend to be located at about the same position (rule of "equilocal heterochromatization"). Instructive examples are afforded by the work of Linnert (1954) on the pachytene karyotypes of species of the plant genus *Salvia* or by Marks and Schweizer (1973) for *Anemone*. The *Cyclops* data confirm this rule: In all 11 chromosomes H-segments are found either at the ends of the chromosomes, as in *C.divulsus*, or at both the ends and the kinetochores, as in *C. furcifer,* or as in *C. strenuus,* dispersed all along the length of the chromosomes in the form of many small segments.

The causes for this uniformity within each karyotype and the manner in which it is brought about during evolution remain obscure. At any rate, selection for uniformity as such must be much stronger than that for any specific type of heterochromatin distribution. Even a chromosomal mechanism as dramatic as that of chromatin diminution seems to be perfectly compatible with almost any type of distribution of H-segments.

Evolutionary changes in the size and location of H-segments may be explained as being due to conventional rearrangements, particularly deficiencies and duplications. However, it is tempting to speculate along a less conventional line with regard to heterochromatin evolution. Britten and Kohne (1969) proposed that blocks of highly repetitive DNA sequences could originate in one step by what they called "saltatory replication". Large gains in the heterochromatin content of a karyotype would thus be achieved in a few steps. In species hybrids of *Nicotiana* (Gerstel and Burns, 1967) the spontaneous formation of large H-segments ("megachromosomes") actually has been observed, and in *Drosophila nasutoides* (Cordeiro et al., 1975) the tiny dot chromosome has acquired a giant second arm which is entirely heterochromatic. Saltatory replication of various sets of interspersed segments of simple sequence DNA could easily have led to the development of widely divergent heterochromatin patterns in the *Cyclops* species.

3. Polymorphism of H-Segments

In all of the three *Cyclops* species investigated here both homologues of a pair often have strinkingly different amounts of germ line limited heterochromatin. In *C. strenuus* this heteromorphism is only generally expressed in the sizes of the chromosomes. In *C. furcifer* and *C- divulsus*, however, homologous H-segments show length differences. Heterochromatin polymorphism of one or a few bivalents is widespread in eukaryotic organisms, for example, in Acridids and other Orthoptera (compare White, 1954; John and Hewitt, 1966), but in the *Cyclops* species, as shown here, this kind of polymorphism is much more pronounced and may extend to the majority of bivalents. The fact that females from widely separated populations show striking similarities in the types of heteromorphic diplotene bivalents present suggests that the observed variability of homologous H-segments is maintained as a component of a balanced polymorphism in the populations. The same holds true for many populations of *C. strenuus* in which heteromorphic bivalents occur along with homomorphic ones in the germ line of females.

The extreme genome dimorphism found in (R3) females clearly can only persist on the basis of the coordinated orientation of all bivalents during meiosis I which has been described in this paper. Mis-orientation of single bivalents has sometimes been observed, but it did not affect the stability of the dimorphism during three years of studying the R3 population. Such errors of orientation would be expected to undergo correction in the next generation.

A striking correlation can be noticed between sex and the type of chromosomes present. All (R3) males were homozygous for the set of large chromosomes (with the occasional exception of one single small chromosome), and more than 90% of the females were heterozygous. This indicates female heterogamety, i.e., the small set of chromosomes must contain a dominant, or epistatic female determining factor, or factors. The reduced chromatin content of the set of small chromosomes cannot as such determine femaleness, since exceptional females occur which are homozygous for the large chromosomes. Such exceptions

could be due to a rare recombination event involving the sex factor. Female heterogamety in Copepods was first found in *Ectocyclops strenzkei* (W. Beermann, 1954), and was later also demonstrated in a number of other *Cyclops* species (Rüsch, 1960).

In organisms with female heterogamety the primary (zygotic) ratio of males to females can vary. This ratio depends upon the orientation of the sex-chromosomes in the Ist division spindle, and the orientation can be influenced by the environmental conditions (Seiler, 1920). The experiments of Metzler (1957) and Rüsch (1960) indicate that this is one of the reasons why there are different percentages of both sexes in Copepods studied under natural conditions. In the population (R3) of *C. strenuus* a strong preponderance of females was always observed. In batches of eggs laid at low temperatures, cytological investigations of early cleavage divisions often revealed a greater percentage of heterozygotes, whereas at room temperature hetero- and homozygosity seem to occur with the same frequency (Table 4).

III. Chromosome Structure and Diminution

1. Arrangement of the Extra DNA in Germ Line Chromosomes

The autoradiographic study of Taylor et al. (1957) on *Vicia faba* represented a first advance towards the elucidation of the molecular structure of eukaryotic chromosomes. The mode of segregation of ^{3}H-thymidine labeled chromatids during two replication cycles was found to conform to the semiconservative type of DNA replication and therefore is consistent with a unineme model of chromosome structure. Further support for this model was provided by the observation that unfixed lampbrush chromosomes could be fragmented by DNAase but not by RNAase or proteases (Callan and Macgregor, 1958), with kinetics expected from the breakage of only one double helix (Gall, 1963). Electron microscopic observations of spread metaphase chromosomes were likewise shown to be in favor of uninemy (DuPraw and Bahr, 1969; DuPraw, 1970). The final proof that the DNA in each chromatid forms only one double helix which runs continuously from one end of the chromosome to the other was provided by the experiment of Kavenoff and Zimm (1973). Using a viscoelastic technique to measure the lengths of very long DNA molecules, these authors found that the length of the longest DNA molecules isolated from *Drosophila* cells was that expected for the largest chromosomes on the basis of their DNA content. Those chromosomes must therefore be unineme with respect to their DNA backbone.

If the chromosomes are unineme, then alterations of their DNA content must be paralleled by changes in the lengths of their DNA axes. This must, in turn, be reflected in the lengths of the mitotic chromosomes and, when the chromosomes are tightly spiralized, in their diameters. If the diameter remains constant, therefore, the length must be directly proportional to the DNA content. This is generally the case in the three investigated *Cyclops* species,

as can be seen from the fact that the lengths of the cleavage chromosomes are considerably different before and after diminution. The situation in the heterozygotes from the population (R3) of *C. strenuus* is especially instructive. In anaphases of the first cleavage divisions, in which the chromosomes are completely isopycnotic, the total length of the heterochromatin-rich set of chromosomes is nearly three times greater than that of the smaller, nearly euchromatic set. A ratio of 2.3:1 was found when the DNA content of the germ line genome and that of the somatic genome were directly determined by Feulgen spectrophotometry.

In meiosis of (R3) females the difference in DNA content between homologues is expressed not as a difference in length but exclusively, or primarily, as a difference in staining intensity and diameter. This condition is maintained until diakinesis (comp. Fig. 22). It is evident that the unequal partners must assume identical lengths in order to pair with each other, since this pairing can only take place between homologous chromosomal segments. Possibly the interstitial germ line chromatin forms loop-like protrusions during the lampbrush-stage of diplotene (comp. Fig. 22b). According to this model, the axes of the paired homologues would consist primarily of the somatic segments which are of equal length in both partners.

It is interesting to observe that the evolution of the somatic portion of the genome apparently proceeds independent from the occurrence and behavior of the germ line limited chromatin. As in other organisms (e.g. in Amphibians, Ullerich, 1965), considerable differences in the size of the somatic genome have been found in the three *Cyclops* species studied here.

2. Structural Aspects of Diminution

The data presented in this paper leave no doubt that prior to diminution the germ line limited DNA forms an integral component of the chromosomes. During diminution, however, interstitial, as well as terminal, DNA segments are removed without disrupting the chromosomes at all. This seems to contradict basic concepts of classical cytogenetics, according to which multiple chromosome breaks and deletions should have serious consequences for the integrity of the genome. While the diminution of a terminal H-segment would only require one break and its subsequent healing, the elimination of interstitial H-segments, as found in *C. furcifer*, would necessitate at least two, and in *C. strenuus* many chromosome breaks and their controlled rejoining. Thus, not only must defined segments be excised from all of the 22 chromosomes, but in order to maintain the linear continuity and the identity of the 22 chromosomes in the soma, the non-eliminated fragments must be correctly joined together again. There is no known parallel to this unique type of DNA elimination in other eukaryotic organisms. The diminution of terminal H-segments in *Ascaris megalocephala* can hypothetically be regarded as a simple breaking-off of the ends, especially since the somatic portions of the chromosomes also simultaneously undergo fragmentation. The elimination of interstitial DNA during the break-down of the giant chromosomes in the macronuclear anlage of *Stylonychia* (Ammermann

et al., 1974) could likewise be interpreted as merely a fragmentation, since there is no subsequent reconstitution of the chromosomes.

One biological event which involves the controlled excision of DNA from chromosomes is that of de-integration of lysogenic phages and other episomes from the bacterial genome (Campbell, 1962). As known from the study of bacteriophage lambda, the integrated prophage genome can be cut out, leaving the host genome intact. Campbell (1962, 1971) proposed a model to explain the mechanisms involved. After induction, the integrated phage DNA is thought to form a loop by the pairing of genetically defined integration or attachment sites. By means of recombination between these sites, the phage genome separates from the bacterial chromosome as a free circle of DNA while the remaining DNA of the host is joined together by the same event.

The mechanism of diminution in *Cyclops* might in principle be identical to that postulated for the reversible integration of bacteriophage lambda. According to this model, the first step in the diminution process would be the arrangement of the germ line limited DNA segments into loops by means of a specific association between adjacent pairs of "elimination sites" (recognition sequences analogous to the attachment sites in bacteriophage lambda). In the second step, the loops are eliminated from the chromosomes in the form of rings. This model enables the non-diminished DNA pieces to be rejoined prior to, or simultaneously with, the removal of the diminuted segments, and makes it possible to excise interstitial H-segments without an effect on the linear continuity of the rest of the chromosome. Since the loss of essential, i.e., somatic, genetic material cannot be tolerated, the mechanism of diminution must work with considerable precision. This precision would primarily depend on the properties of the hypothetical elimination sites.

The size of the chromatin, or DNA, fragments excised by the diminution process is not known. However, it is reasonable to assume that these fragments are smaller than entire H-segments in *C. divulsus* and *C. furcifer*, and probably also in *C. strenuus*. In the first two species the eliminated chromatin first becomes visible in the form of numerous very small particles dispersed throughout the nucleus in the early prophase of the elimination division. Only as the division proceeds do the particles merge into the larger more conspicuous ones which fill the spindly during meta- and anaphase. The slightly different course of events in *C. strenuus* can be interpreted as fusion of the chromatin particles into larger ones already in interphase ("dotted nuclei", cf. Fig. 29a). The assumption that the germ line chromatin, even if present in large blocks in *C. divulsus* and *C. furcifer*, consists of numerous small subunits which are individually excised may also help to understand the special situation in *C. furcifer*, where the diminution process spreads over two consecutive division cycles. This has already been discussed in detail in an earlier report (S. Beermann, 1966).

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