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Underreplication of a Polytene Chromosome Arm in the Chironomid *Prodiamesa olivacea*

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Abstract. The genome of *Prodiamesa olivacea* (Diptera, Chironomidae) has a 2C DNA content of 0.25 pg. Mitotic metaphases reveal 3 pairs of chromosomes: 2 metacentric ones and one submetacentric. The latter comprises 20.8% of total Feulgen DNA. During larval polytenization the complemental portion of the 3rd falls to 6.5%. Concomitantly the polytene 3rd chromosome is much shorter than expected. It has no constriction and is shaped like a ball sector. – Underreplication is understood as suppression of DNA syntheses mainly in the long arm of the 3rd chromosome at the first to third endoreplicative cycle. Most of the dense heterochromatin seen in the apex of the 3rd polytene element is not itself underreplicated; it conceals the underreplicated long arm of this chromosome. – In ovarian nurse cells which are closely connected with the germ line the longer heterochromatic arm of the 3rd polyneme chromosome is fully replicated. – Underreplication is discussed in the context of "DNA silencing".

I. Introduction

Prodiamesa olivacea Meigen 1818 is a member of the nematoceran family Chironomidae. The subfamily Diamesinae shares with the other six subfamilies (Goetghebuer, 1936, 1939) karyotypes with only a small number of chromosomes (n=2 to 6), and they present cytologically excellent polytene chromosomes (Bauer, 1935, 1936). In *Prodiamesa* salivary gland nuclei a chromocenter connects 4 long arms. A tiny 5th element with a peculiar concentric banding never sticks to the chromocenter, although it manifests a dense heterochromatic knob. On the basis of the polytene situation it is impossible to decide whether there are 3, 4, or 5 chromosomes (Bauer, 1936). Bauer and Beermann (1952) mentioned that spermatogenesis takes place with n=3 chiasmatic bivalents; but later *Prodiamesa* was cited as possessing 5 chromosomes (Beermann, 1962).

The aim of the present study was to definitively establish the karyotype and elucidate the structure of the small polytene element by cytological and

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cytophotometric comparison of polytene, polyneme and mitotic chromosomes. When underreplication had been observed for the first time in a chironomid midge special attention was paid to its extent and progress.

II. Material and Methods

Animals. Prodiamesa olivacea is a member of Diamesinae species (family Chironomidae) inhabiting European plains (Goetghebuer and Lenz, 1939). The insects were collected as larvae from brooks in the Swabian Jura and the Black Forest or were obtained from a shop for fish food in the Rhineland (Matwew, Heinsberg-Oberbruch, F.R.G.). The larvae survived in bowls of tap water at 4–10° C. Under these conditions only a fraction of pupae developed into adults. Breeding did not succeed in the laboratory. Because larvae were selected according to their body size (ca. 13 mm; last instar) it is probable that mainly females were used.

The DNA content of *Prodiamesa* nuclei was calibrated with nuclei of red blood cells from the same hen (Weiße Hybride, Henry farm, Tübingen).

Tissue Preparation. Salivary glands, Malpighian tubules and hind gut were dissected in a medium after Robert (1971) which contained no magnesium. Fixation was performed in ethanol-acetic acid (3:1) for 1 min.

In order to determine DNA contents, brains and gonads were prepared in a hypoosmotic solution of 0.95% sodium citrate and 0.25% Triton X 100, afterwards incubated in this medium on a siliconized slide for 60 min, and then transferred to an untreated slide. Hen blood was diluted with 4% sodium citrate (ca. 1:20) and 5 µl smeared next to the squashed *Prodiamesa* tissue. Slides were air dried and fixed in methanol for 1 min.

Cytophotometry. The absorption values in arbitrary units (AU) were determined with double-beam universal microspectrophotometers (UMSP, Zeiss, Oberkochen; Caspersson et al., 1955; Trapp, 1966). The Feulgen slides were scanned near maximal extinction (560 nm). For polytene, polyneme and metaphase chromosomes an analog UMSP, and for nuclei of ganglia, follicle envelopes, sperms and hen erythrocytes a digital UMSP was used, both with ultrafluar objectives 100/1.25 and projectives 100:1. The electric current of the Xenon lamp was stabilized at 27 amps. The smaller measuring diaphragm (0.5 µm diameter at the plane of the specimen) yields 4 times higher values (AU_{05}) than the larger one (1.0 µm diameter; AU_{10}). The precision of the analog UMSP, to which the digital one is similar, was checked with repeated readings of the 3rd polytene chromosome (mean relative error $1.9 \pm 0.2\%$, maximum 6.8%; $n = 31$) and by recording of transmission.

The determination of chromosomal equivalents were designed to spare time and gain results from complements where not all polytene elements are accessible to microphotometry. Here the absorption values of 3rd chromosomes were projected by scanning onto the distal left arm of 1st chromosomes in the same nuclei. With the aid of this method total band number was calculated and the relative DNA contents of many 3rd chromosomes in some salivary glands were registered.

Detailed procedures are described in Zacharias (1977).

Autoradiography. To detect DNA synthesis, 20 salivary glands were incubated 30 min at 25° C in 250 µl modified medium after Robert (1971) which contained 125 µCi ^{3}H-deoxythymidine (23 Ci/mM; Amersham Buchler, Braunschweig-Wenden). Further processing followed Pelling (1964); the exposure required 20–25 d.

Staining Methods. Chromosomes were diagnosed after 30–60 min staining with lactic acetic orcein (Beermann, 1952) or carmine and orcein acetic acid (Pelling, 1964). The fluorescent bibenzimide derivative H 33258 (Hoechst, Frankfurt M., courtesy of Dr. Loewe) was applied after Seth and Gropp (1973).

For microphotometry, the chromosomal DNA was Feulgen stained. Squashes were postfixed in ethanol and 37% formaldehyde (4:1); they were stored in propanol-(2) at −20° C. The 0.7 mm thin slides, passed through a descending ethanol series and aqua dest., were exposed to 1 N HCl

at room temperature for 1 min. As a rule, DNA depurinating hydrolysis was performed in 5 N HCl at 25° C (Jordanov, 1963) for 60 min (comp. Fig. 1); reaction was stopped with 4° C aqua dest. Salivary glands were hydrolysed 90 min in 1 N HCl at 40° C (Crouse and Keyl, 1968; Meer, 1976) to reduce optical density of polytene heterochromatic structures. The time course of DNA hydrolysis in HCl with variing ionic concentrations was demonstrated in Zacharias (1977). Slides were Feulgen stained as reported by Sokoloff and Zacharias (1977) and were stored in the dark at 4° C to prevent fading (Speiser, 1973).

III. Results

A. DNA Content of the Basic Genomes

Mitotic metaphases are not appropriate for the determination of absolute DNA values because good squashes of the chromosomes cannot be obtained in the absence of acetic acid. Acid treatment prior to the Feulgen hydrolysis is not desirable since even weak acids considerably degrade DNAs (Sederoff et al., 1975; Hirschi, 1977). This consideration led to the choice of the haploid sperms and the mainly diploid neuroblasts.

In order to determine the DNA content of brain nuclei, two series of slides were subjected to a fractionated hydrolysis. Data points were taken from duplicate slides with hen blood cells as standard. The means of the 30 examined specimens of each group were plotted in Figure 1.

By convention, one compares the sample with the DNA standard at maximum absorption (Ris and Mirsky, 1950). It was expected that optimal staining correlates with proportional binding of pararosaniline sulfureous acid to the DNA of different nuclei. The theory of deoxynucleoprotein hydrolysis was further developed by Rasch and Rasch (1973) who have analysed the variable resistance of DNA in chromatin to acids. They suggest that the kinetics of chromatin hydrolysis result from at least two DNP fractions which are converted at different rates into Feulgen stainable substance. Therefore, only those depurinating processes to which the different DNP fractions contribute proportionally are immediately comparable; with hen erythrocytes for example the required hydrolysis lasts 7 h. The complications can be disregarded if the kinetics of sample and standard diverge from each other at random. That this is the case in our essay was checked by analysis of variance of paired quotients of extinction values from erythrocytes and brain nuclei. They are randomly distributed about the mean. Hence the nuclei of hen erythrocytes and *Prodiamesa* neuroblasts can be compared at all available time-points.

The diploid DNA content of *Prodiamesa* turns out to be approximately one tenth that of hen erythrocytes. The DNA content of the latter is 2.5 pg (Rasch et al., 1971), thus a tentative 2 C value of 0.261 pg DNA can be calculated for *Prodiamesa olivacea.*

The relative errors of erythrocyte samples were distinctly smaller than those of the midge tissue. Mature hen erythrocytes do not synthesize DNA, whereas larval midge ganglia do. About 15% of brain nuclei were labelled after exposure to ^{3}H-deoxythymidine; this agrees with 16.4% in *Drosophila hydei* (Berendes and Keyl, 1967). On the other hand, the degree of data dispersion indicates that the brain nuclei measured do not include any with 4 C DNA content.

The fact that few brain nuclei replicate suggests that the true DNA content of diploid *Prodiamesa* nuclei lies at the lower limit of confidence. If $\mu = \bar{x} - t \times s_{\bar{x}}$, where the degrees of freedom for t are 22 (n = 23; comp. Fig. 1) and the probabil-

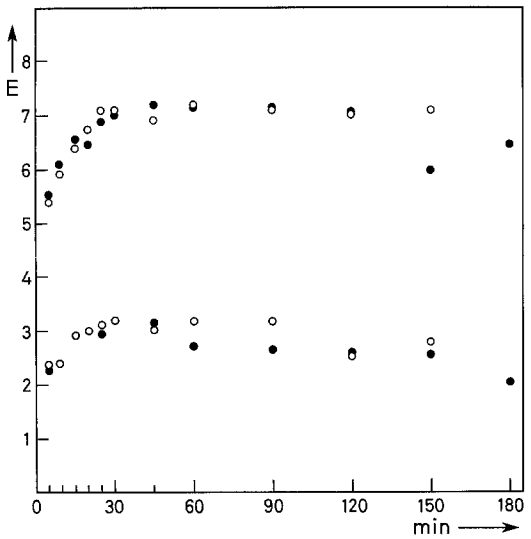

Fig. 1. Kinetics of Feulgen hydrolysis in hen erythrocytes (top) and *Prodiamesa* neuroblasts (bottom); 5 N HCl at 25° C. Open and closed circles indicate two different Feulgen series, representing 30 nuclei, each scanned once with digital UMSP. The 0.5 µm diaphragm which was used with 2 C-nuclei of *Prodiamesa* yielded 4 times higher extinctions than the 1.0 µm diaphragm used with hen erythrocytes (2.5 pg DNA; Rasch et al., 1971). The DNA content of diploid *Prodiamesa* nuclei was 0.25 pg

ity of error is set at 0.01, the 2 C value of *Prodiamesa olivacea* turns out to be 0.247 pg DNA.

This was confirmed by absorption measurements of sperm nuclei. Each sample comprised 30 specimens. After 30 min hydrolysis sperms showed 1.26 AU_{05}, hen erothrocytes of the same slide 6.94 AU_{10}; the data after 60 min hydrolysis were 1.32 AU_{05} and 7.53 AU_{10}. These data correspond to 1 C levels for *Prodiamesa* nuclei of 0.113 pg and 0.109 pg DNA, respectively.

B. The Mitotic Karyotype

1. Cytology

In view of the presence of a chromocenter in giant polytene chromosomes one can assume that the dense material in mitotic interphase nuclei is also due to adherence of chromatids in the kinetochoric region. A single nucleolus is located adjacent to this chromocenter. In *Prodiamesa olivacea* well analysable division figures of mitoses were found in larval brains of the last instar and in ovarian follicle cells from pupae (method Leoncini, 1977). The mitotic karyotype comprises 3 pairs of chromosomes which are not uniform in length (Fig. 2).

Seven photographs of (pro-) metaphases from neuroblasts and twenty three from follicle cells were available for length determination. The absolute lengths of metaphase chromosomes in follicles were about 4.0 µm (1st), 2.3 µm (2nd) and 1.9 µm (3rd). Figure 3 shows the chromosomal idiograms of both tissues

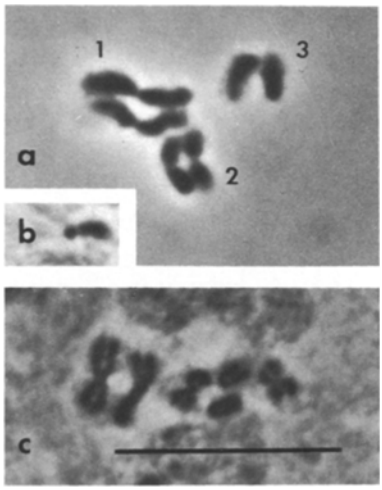

Fig. 2a–c. Sets of mitotic chromosomes from larval brain (**a**) and follicle cells of pupae. **a** Metaphase. The pairing of the homologues is obvious. **b** Chromosome 3 in metaphase with primary constriction. **c** Late metaphase with separated chromatids. Stained with lactic acetic orcein. Bar = 10 μm

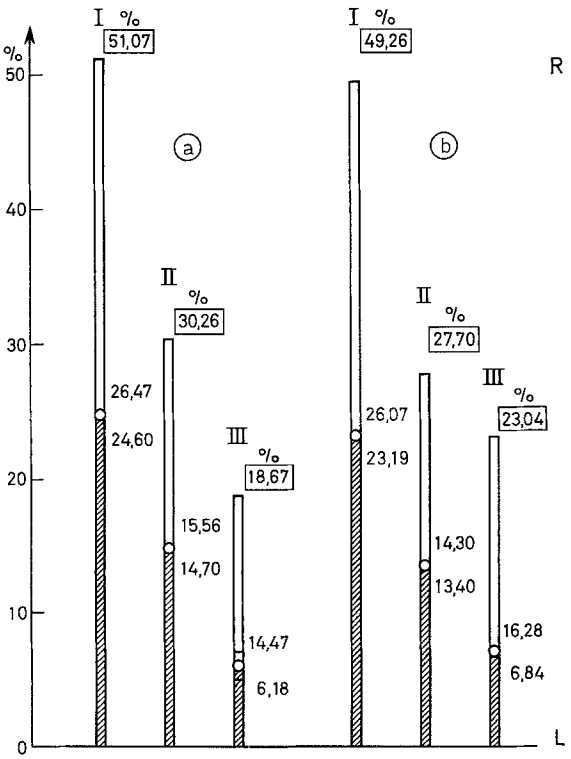

Fig. 3a and b. Idiograms of mitotic chromosomes. **a** Brain metaphases (n = 7) from last larval instar. **b** Metaphases of follicle cells (n = 23) from pupae. Percentage values represent mean portions of the total lengths in the complements. *R* Right arm, *L* shorter left arm (hatched). The circles mark kinetochore positions

Table 1. Extinction values of chromosome pairs in metaphases from Feulgen stained follicle cells. Double readings with analog UMSP; 0.5 µm diaphragm. In the first nucleus only the chromosome pairs 1 and 3 were determined; the value of the 2nd chromosome was calculated (*). The difference between total metaphase absorption and sum of single scanned chromosomes indicates precision for measuring field setting in each nucleus

Nucleus	1st chromosome		2nd chromosome		3rd chromosome		Sum of chromosomes AU	Total metaphase AU	Difference AU
	AU	%	AU	%	AU	%			
1	17.90±0.30	49.93	10.55*	29.43	7.40±0.30	20.64	–	35.85±0.85	–
2	16.45±0.05	51.01	8.85±0.55	27.44	6.95±0.15	21.55	32.25	30.80±0.80	1.45
3	15.80±0.15	50.80	8.80±0.20	28.30	6.50±0.10	20.90	31.10	30.90±0.30	0.20
4	–		–		6.30±0.10	20.39	–	30.90±0.80	–
5	18.45±0.35	52.27	9.60±0.20	27.19	7.25±0.25	20.54	35.30	34.25±0.45	1.05
6	–		9.50±0.30	28.19	–		–	33.70±1.00	–
7	16.80±0.10	53.76	7.95±0.05	25.44	6.50±0.20	20.80	31.25	30.60±0.80	0.65
8	–		–		6.70±0.30	20.81	–	32.20±0.50	–
9	16.10±0.60	51.85	–		–		–	31.05±0.95	–
Mean of complemental portion		51.60		27.67		20.80			0.84
Standard error of mean		0.55		0.55		0.14			0.27
Coefficient of variation[a]		0.03		0.05		0.02			0.64

[a] Coefficient of variation = standard deviation : mean

using the relative means. In brains the 1st chromosome amounts to 51.1%, the 2nd to 30.2% and the 3rd to 18.7% of the total complement. The corresponding data from follicle cells were 49.3%, 27.7% and 23.0%.

The 1st and 2nd chromosomes have kinetochores in median position. The arm-proportions were 1.06 in the 1st and 1.12 in the 2nd chromosome. The longer chromosome arm was designated as right (R), the shorter left (L). The small 3rd chromosome often appears undivided; in about 30% of all metaphases a primary constriction places the kinetochore in a submedian position (Fig. 2b).

The mean quotient of long to short arm is 2.38 in follicle cells; only two quotients were obtained from the few neural metaphases (2.54 and 2.19). Aside from kinetochoric segmentation, no further constriction or heterochromatic condensation was seen with any of the staining procedures used.

The relative length values of the 3rd chromosome from neuroblasts (18.67%) and from follicle cells (23.04%) are apparently correlated in inverse ratio to the longer chromosomes. This difference in 3rd chromosome contraction is greater than random. The result means that the metaphasic 3rd chromosome is about 5% more condensed in brains than in follicles. It was not possible to decide whether this kind of variation is exclusively due to the right arm of the 3rd chromosome.

2. DNA Photometry

Nine well squashed follicle metaphases of one Feulgen series were scanned twice with the analog UMSP (Table 1). The chromosomes share the complement in the proportions 51.6% (1st), 27.6% (2nd) and 20.8% (3rd).

The accuracy of these measurements is indicated by the modest scatter of dublicate readings. It was further controlled by comparing the sum of extinction values of the three pairs of chromosomes with the extinction of the total metaphase. The sum of the single determinations does not deviate significantly from the overall measurement of the same metaphase.

C. The Polytene Karyotype

1. Cytology

The salivary glands of *Prodiamesa* larvae are conspicuously dimorphic (Melland, 1941; Zacharias, 1977). Although the number of their giant cells is not strictly fixed there is a significant difference: in the right gland 37 ± 3 cells, in the left one 44 ± 1 were counted independently three times ($n = 11$ in each case). Whether a smaller number of cells is compensated by a higher level of polyteny in comparable phases of development was not determined.

The long arms of the polytene complement are linked in a chromocenter (Fig. 4). But sometimes the longitudinal continuity of giant chromosomes is so disturbed in squashes that the chromocenter cannot be established. Not knowing the mitotic complement one may put together any long arm with

each remaining long arm to a complete chromosome, or one may arrive at n=5 by counting each arm independently (Bauer, 1936).

The small chromosome usually does not join in the chromocenter, although it exhibits a distinct heterochromatic clot. In squashed salivary cells and in nuclei opened by micromanipulation the highly polytene small chromosome separates easily from the remaining elements. Only in a few cases a thin thread of chromatin was seen to connect the heterochromatic knot and the chromocenter.

In the metaphase complement the 1st chromosome is almost twice as long as the 2nd, both are metacentric and thus different from the 3rd (comp. Fig. 3). It was assumed that the possible discontinuities of polytene chromosomes coincide with the kinetochoric regions of the mitotic chromosomes.

The hypothesis which combines the two long arms into the 1st polytene chromosome and the two short arms into the 2nd was examined statistically. The mean of 30 quotients of the two long metaphase chromosomes was 1.77 (standard deviation SD=0.16), the mean of 25 quotients from salivary gland chromosomes derived with the hypothesis was 1.78 (SD=0.21). These means are not significantly different (p=0.05).

If the two long polytene arms belong together and form the 1st chromosome, the 2nd polytene chromosome must comprise the two shorter arms of the complement. The small chromosome in salivary gland nuclei is homologous with the 3rd of metaphases.

The pairing of the polytene chromosomes is always very close. Short gaps are only seen in connection with heterozygous inversions. Unpaired regions up to total separation were observed in the small 3rd chromosome.

2. Structural Details of the Salivary Gland Chromosomes

The 1st Chromosome. The right arm of the 1st chromosome is the longest element within the polytene nucleus. Towards the kinetochore it is terminated by heterochromatin either as wide as the whole structure or tapering off. The distal end also shows heterochromatic bundling and is sometimes shaped as a button. An inversion is frequently found in the middle of this arm which shows a figure eight in heterozygotes (Fig. 4). A highly expanded puff near the chromosomal end is the most conspicuous active site of this arm.

The left arm of the 1st chromosome contains a heterochromatic constriction about 35 bands from the distal end (Fig. 5a, b). Directly adjacent to the kinetochore the sole nucleolus is formed, spotted with lumps of dense material. In the salivary glands a Balbiani ring (BR 1) is found adjacent to the nucleolus. Because of the proximity of these two structures the BR is barely discernible, by analogy to the situation described for *Acricotopus lucidus* (Mechelke, 1935; Panitz, 1972). A clear distinction has been achieved with selective ^{3}H-uridine incorporation (Fig. 5c) and a fortunate squash which shows the nucleolus linked to 1 L merely with a chromatin thread (Fig. 5d). A paracentric inversion less frequent than that which characterizes the right arm occurs in the middle of the left arm. Beyond the first third of the left arm (seen from the chromosomal

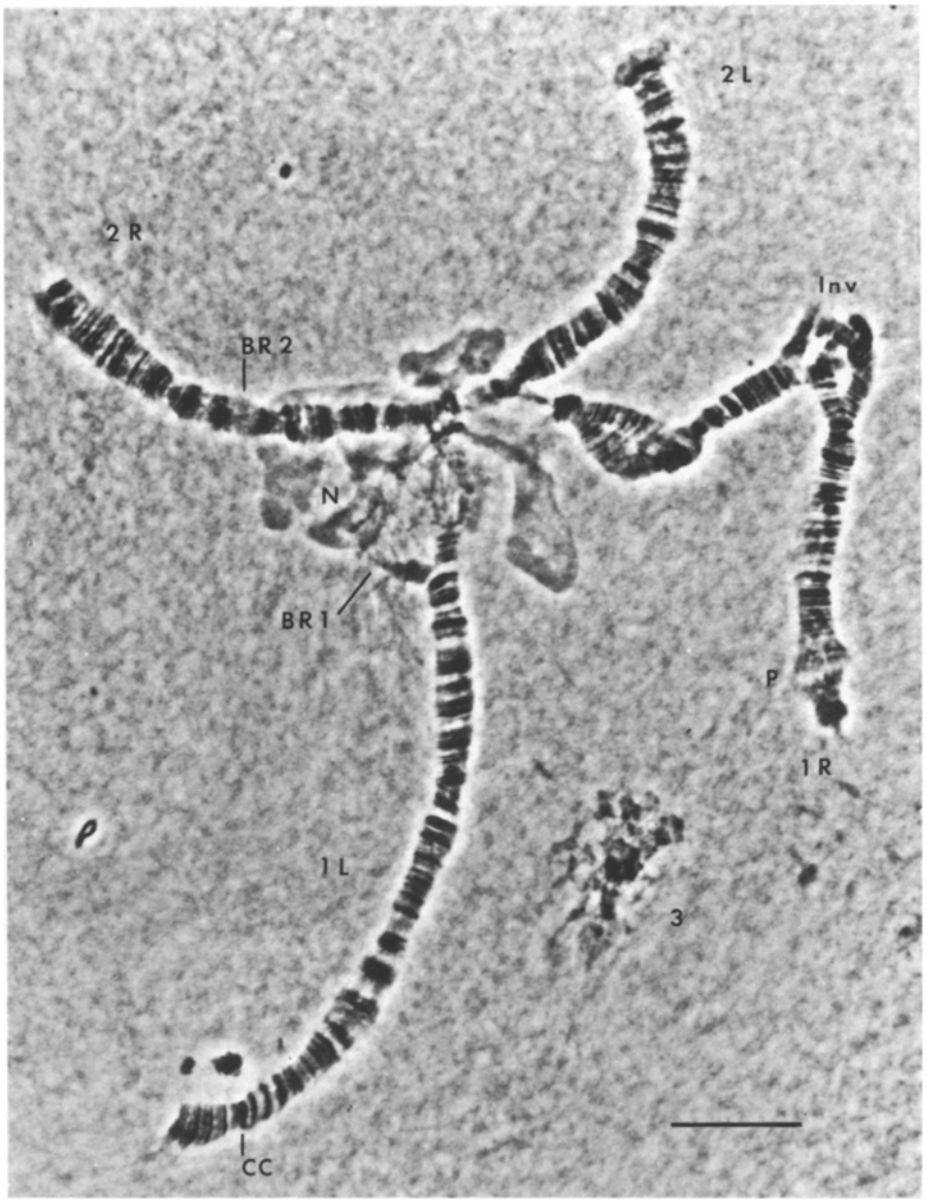

Fig. 4. Salivary gland chromosomes from last larval instar. The 3rd chromosome does not join the chromocenter. *BR* Balbiani ring, *CC* constriction complex, *Inv* heterocygous inversion, *N* nucleolus, *P* prominent puff, *R, L* right and left chromosomal arms. Carmine-orcein acetic acid. Bar = 50 μm

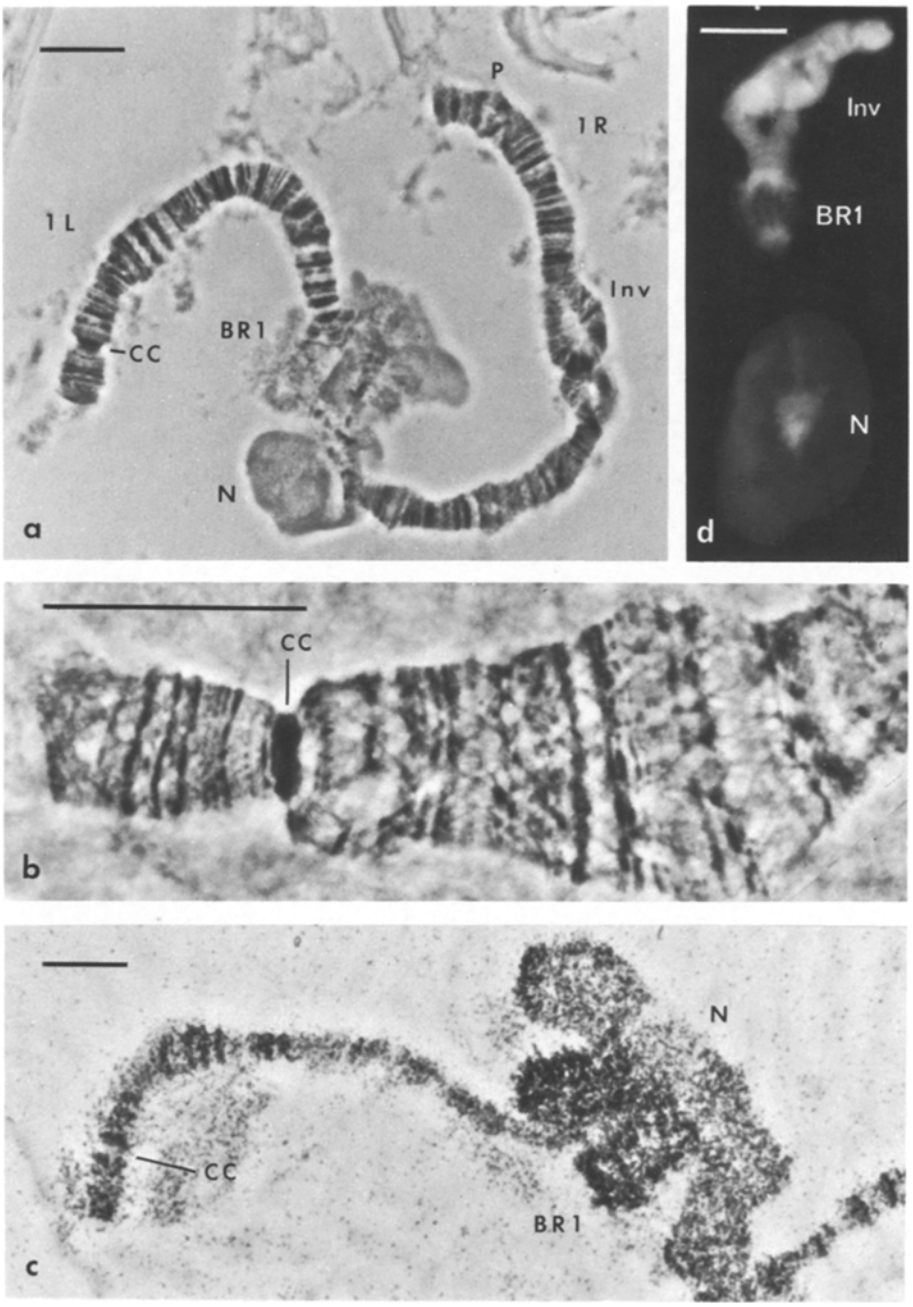

Fig. 5a–d. Chromosome 1 from larval salivary gland nuclei. **a** Survey. Carmine-orcein acetic acid. The slide was made available by courtesy of Dr. Pelling. **b** Distal end of the left arm shows a constriction complex. Carmine-orcein acetic acid. **c** Discrimination between Balbiani ring 1 and nucleolus by different ^{3}H-uridine incorporation after 30 min. Unstained autoradiogram. **d** Nucleolus and its organizer separated from BR 1 by a chromatin thread. H 33258 fluorescence. Abbreviations see Fig. 4. Bars = 20 μm

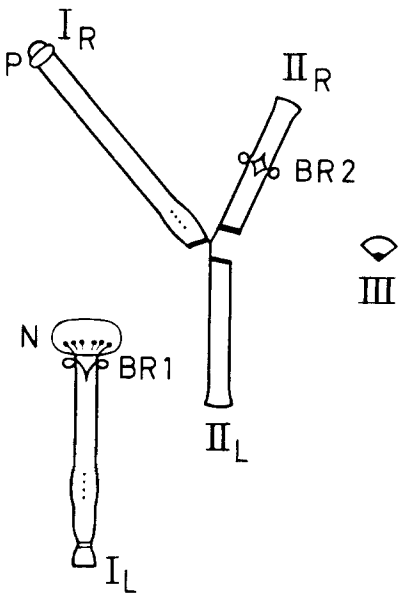

Fig. 6. Schematic presentation of the polytene set of salivary gland chromosomes. The left arm of the 1st is very often detached from the chromocenter. The dotted lines indicate weakly paired regions. Abbreviations see Fig. 4

end) a localized swelling often indicates weakened lateral association of chromatids (Fig. 5b). Sometimes the homologues do not pair at all at this site (in the absence of any obvious structural heterozygosity). A similar place of weak pairing and chromatid association is very common in the kinetochoric region in the right arm of this chromosome.

The 2nd Chromosome. A feature of this element is the formation of terminal fans. The bond linking the two arms in the kinetochoric region is stronger than that of the 1st chromosome. Thus, as a rule, the 2nd chromosome is preserved as a unit after squashing, while one arm of the 1st, usually the left, is detached from the chromocenter (Fig. 6).

As in metaphases, the two arms of the 2nd are approximately equal in length. In the absence of a prominent banding marker the element which bears a further Balbiani ring in the middle (BR 2) was defined as the right arm. This structural modification is not always fully developed in salivary cells.

The 3rd Chromosome. As compared with the 3rd chromosome in metaphase the salivary 3rd seems to lack one arm. A euchromatic banded section simply appears topped by a heterochromatic knob (Fig. 7b). Without the mitotic standard for comparison, the element gives the impression of an originally acrocentric chromosome. Bauer (1936) interpreted the chromomeric pattern as a succession of concentric spherical shells so that the entire element should be ball shaped (comp. Fig. 7a). However, most structural variants of the 3rd chromosome can be best understood as representing ball sectors (Fig. 7c) which open as fans with angles of more than 100° after squashing. Similar fans are not uncommon at the ends of giant chromosomes (Beermann, 1962). In *Prodiamesa* the heterochromatic clot bunches the base of the fan; the 3rd polytene chromo-

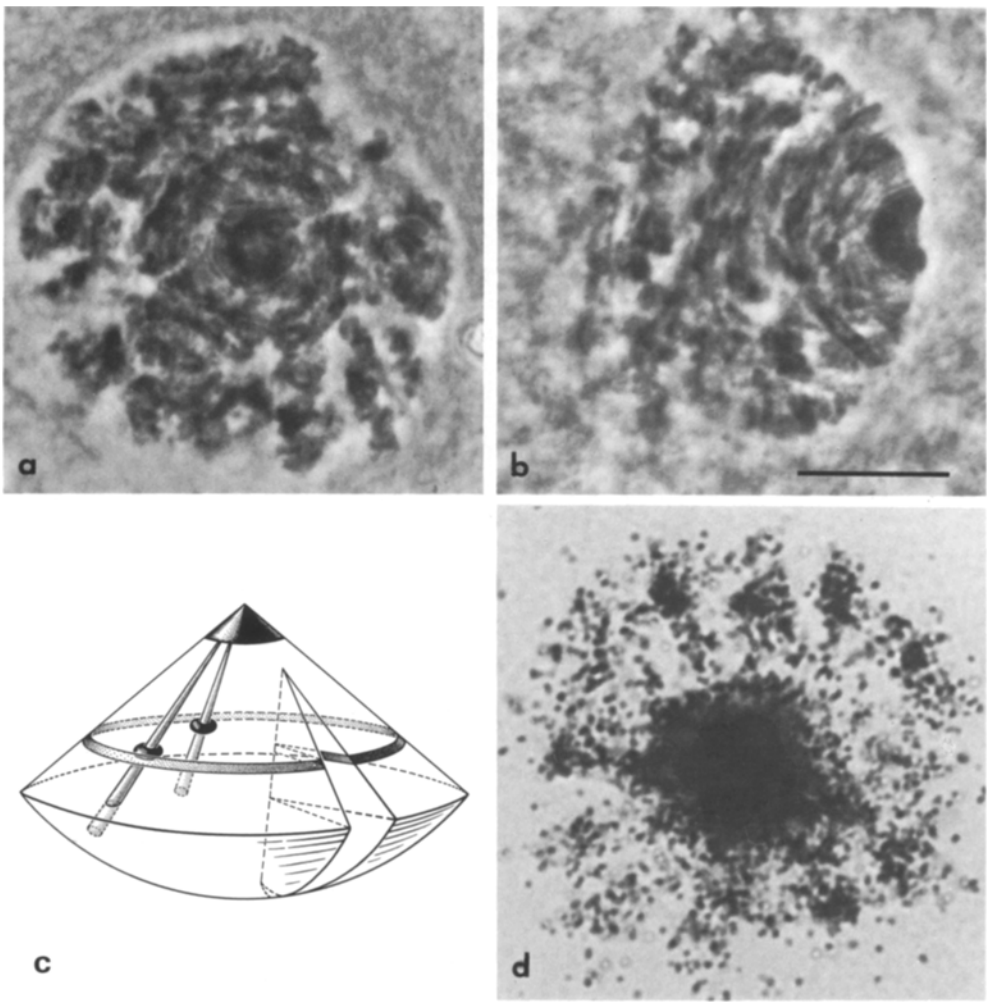

Fig. 7a–d. Chromosomes 3 from larval salivary gland nuclei. **a** Concentric bands surround the heterochromatic knob; slide by courtesy of Dr. Pelling. **b** Side view. The polar knob marks the kinetochoric region and contains the underreplicated right arm of the chromosome. **c** Idealized structure. The positions of two chromatids in the left arm are shown, each with a chromomere. The knob mainly conceals polytenized β-heterochromatin which hides the underreplicated right arm (α-heterochromatin). **d** Autoradiogram after 30 min ^{3}H-thymidine incorporation. The knob is clearly labelled. **a, b** Carmine-orcein acetic acid, **d** unstained. Bar = 10 μm

some may be regarded as its own end. This idea was substantiated by focus series.

3. Number of Bands

The total number of bands was estimated by extrapolation of a known euchromatic area to the whole chromosomal set. Extinction equivalents of the 3rd

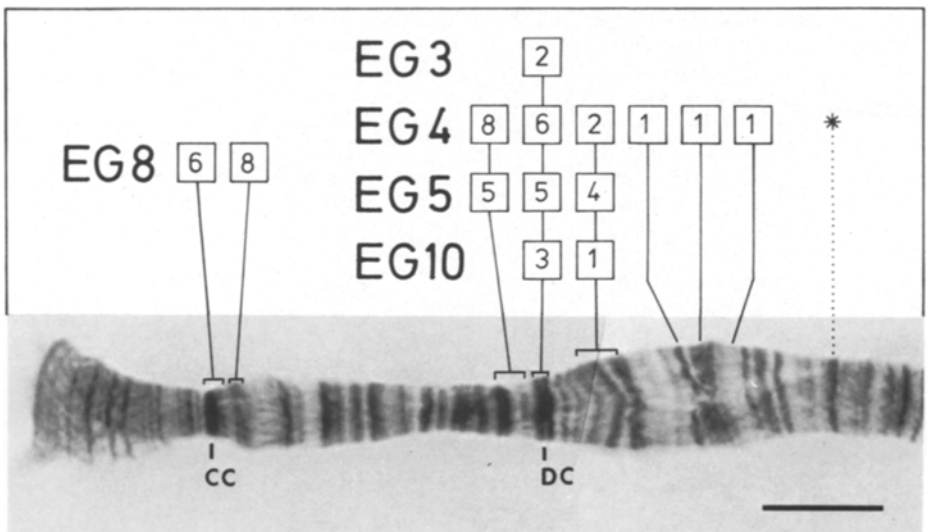

Fig. 8. Indirect determination of band number (see p. 34) and exceptional DNA contents of 3rd chromosome (see p. 40). Only slides of Feulgen series *EG* were used. Numbers in squares count UMSP measurements, some of them are double readings. *CC* Constriction complex, *DC* double complex of bands in chromosome 1 L. The single extreme 3rd with a complemental portion of 9.1% shows the largest equivalent (*). Bar = 10 µm

chromosome were projected to the left end of the 1st in the same salivary gland nucleus. For this purpose only 3rds representing 6.5% of the complement were used (comp. p. 40). The area of the 1st chromosome which is equivalent to the whole 3rd was most frequently marked off by a double complex of bands (DC) as mapped in Figure 8 (slides *EG 3, 5, 10* only!). Results from 20 nuclei were transferred to a 1st chromosome with a well resolved banding pattern, and the bands were counted three times independently with three repetitions in each case: 80 ± 2.5 bands are equivalent to 6.5% of the complement. A total set of giant chromosomes therefore should comprise approximately 1,230 bands.

4. Relative Lengths of Salivary Gland Chromosomes

The lengths of 13 sets of highly polytene chromosomes, selected for lack of squashing artifacts, were sampled. Where the 3rd was of radial appearance the radius was determined. The average length of the 1st chromosome was 412 ± 32 µm or 61.0%, of the 2nd 246 ± 25 µm or 36.5% and of the 3rd 17 ± 2 µm or 2.5%. These data compared with those from metaphase chromosomes indicate a shift in the complemental proportions which must be attributed solely to the 3rd chromosome because the ratio of 1st to 2nd chromosome is constant (1.8; p. 30). On the other hand, the salivary gland chromosomes 1 and 2 are about 100 times longer than the highly condensed corresponding elements in metaphases. This stretching factor is barely 50 in *Chironomus tentans,* whereas

Table 2. Extinction values of Feulgen stained polytene chromosomes from salivary glands. Analog UMSP; 1.0 µm diaphragm. Normally only 3rd chromosomes were repeatedly scanned. Nuclei

Nucleus	1st Chromosome		1 R		1 L	
	AU	%	AU	%	AU	%
1	1,237.0	60.61	691.0	33.86	546.0	26.75
2	1,384.0	63.12	758.0	34.57	626.0	28.55
3	1,515.0	58.65	801.0	31.01	714.0	27.64
4	1,709.5	60.07	921.0	32.36	788.5	27.71
5	2,107.5	60.12	1,101.0	31.41	1,006.5	28.71
6	2,445.0	61.84	1,335.0	33.77	1,110.0	28.07
7	3,133.5	60.33	1,658.5	31.93	1,475.0	28.40
8	3,719.5	59.88	2,055.0	33.08	1,664.5	26.80
9	4,113.0	59.17	2,445.5	35.18	1,667.5	23.99
10	4,471.5	58.64	2,511.5	32.94	1,960.0	25.70
11	4,490.0	57.80	2,408.0	31.00	2,082.0	26.80
12	4,768.5	60.31	2,653.0	33.55	2,115.5	26.76
Mean of complemental portion		60.05		32.89		27.16
Standard error of mean		0.42		0.40		0.39
Coefficient of variation		0.02		0.04		0.05

in *Drosophila melanogaster,* it is 150 (Beermann, 1952; Bridges, 1935). The ratio of width to length of the 2nd polytene chromosome is about 1:20 in *Prodiamesa* and agrees with *Ch. tentans.*

The 3rd polytene chromosome differs extremely from the two larger ones: its factor of stretching is only 10 compared with metaphase.

5. Relative Amounts of Feulgen DNA in Salivary Gland Chromosomes

Three groups of altogether 27 salivary gland nuclei with highly polytene chromosomes were measured with the analog UMSP. In the first sample only the relative DNA contents of the 3rd chromosomes were determined ($n=10$); a second sample recorded all three chromosomes separately in 5 nuclei. These data were not significantly different from those of a third sample ($n=12$) which evaluated individual chromosome arms (Table 2); the following mean contents of Feulgen DNA were found: 60.0% (1st), 33.5% (2nd) and 6.5% (3rd). If one considers all three samples, the 3rd contains 6.3% on average. As with the length values, quotients were formed with the extinction values of chromosome 1 and 2. The mean of 5 quotients of the two long metaphase chromosomes was 1.88 ($SD=0.16$; Table 1); the mean of 17 quotients of the corresponding giant chromosomes was 1.79 ($SD=0.08$). The difference as judged by the t-test

2 and 11 possess 3rd chromosomes with significantly extreme relative DNA content

2nd Chromosome		2 R		2 L		3rd Chromosome	
AU	%	AU	%	AU	%	AU	%
672.0	32.92	349.0	17.10	323.0	15.82	132.0	6.47
734.5	33.50	349.5	15.94	385.0	17.56	74.2	3.38
914.0	35.38	464.0	17.96	450.0	17.42	154.3	5.97
950.5	33.40	482.0	16.94	468.5	16.46	185.8	6.53
1,160.0	33.09	595.0	16.97	565.0	16.12	238.0	6.79
1,244.5	31.48	601.5	15.22	643.0	16.26	264.2	6.68
1,736.5	33.44	827.5	15.94	909.0	17.50	323.7	6.23
2,113.5	34.02	1,023.5	16.48	1,090.0	17.54	378.8	6.10
2,364.0	34.01	1,186.0	17.06	1,178.0	16.95	474.0	6.82
2,591.8	33.99	1,282.0	16.81	1,309.8	17.18	562.0	7.37
2,571.0	33.10	1,260.0	16.22	1,311.0	16.88	707.2	9.10
2,654.5	33.58	1,309.5	16.57	1,345.0	17.01	483.0	6.11
	33.49		16.60		16.89		6.46
	0.26		0.21		0.17		0.37
	0.03		0.04		0.04		0.20

is not significant ($p=0.05$) so that the means of the two samples can be regarded as equal. It is clear that the change in complemental proportions from the mitotic to the polytene state is brought about by the altered DNA content in the 3rd chromosome. This 3rd is not only partially underreplicated in the polytene condition but is also less extended than the two other elements. This appears in the ratio of its mean DNA portion to its mean length portion ($6.5\% : 2.5\% = 2.6$).

Constant Proportion of the 3rd Chromosome During the Last Larval Instar. Several inferences concerning time and extent of underreplication can be made from the photometric data on 3rd polytene chromosomes of the last larval instar. In particular one can decide whether this element progressively excludes more and more of its DNA from replication or whether a part of it always participates in regular DNA syntheses. To this end the extinctions of the 27 salivary gland nuclei were roughly standardized against 4 of the smallest interphasic brain nuclei. It turns out that the polytene nuclei belong to the 9th, 10th, 11th and 12th cycle of endoreplication. An analysis of variance ($p=0.05$) proved that the large majority of 3rd chromosomes from these nuclei has the same relative DNA content. The exceptional values 3.4% and 9.1% will be discussed on p. 40.

The relative decrease of the 3rd from a metaphasic portion of 20.8% obvi-

ously approaches a plateau of 6.5% in the highly polytene complements. This suggests that underreplication does not occur in late endoreplications. Furthermore, it allows one to calculate the fraction of the 3rd chromosome which is not subject to underreplication.

Underreplicated Component of the Genome. Given that the relative DNA content of an underreplicated polytene chromosome is constant during the last DNA syntheses, and given its relative DNA content before underreplication, it is possible to calculate its non replicating fraction. For *Prodiamesa* the answer is obtained by solving the following expression for x.

$$\frac{100[2^n(III-x)+2^u x]}{2^n(R+III-x)+2^u x} = 6.5\,\%$$

x is the unknown DNA portion of the *metaphasic* chromosome 3, which does not endoreplicate;
III is the relative DNA content of the entire 3rd chromosome in metaphase (20.8%);
R is the relative DNA content of the remaining metaphase chromosomes 1 and 2 (79.2%; R+III=100%);
2 is the factor of reduplication;
n is the number of completed endoreplications;
u is the number of endoreplications during which the portion x of the 3rd chromosome takes part in DNA syntheses;
6.5% is the constant complemental portion of the highly polytenized 3rd chromosome.

At a certain stage of endoreplication the DNA content of a 3rd chromosome is $2^n(III-x)+2^u x$; the DNA content of the whole nucleus amounts in the same stage to $2^n(R+III-x)+2^u x$. All expressions are based on the simplified supposition that underreplication is a single step event.

The desired DNA fraction of the 3rd mitotic chromosome which will not be (totally) polytenized amounts to x=15.3% of the genome. This means that the fully replicating portion of the 3rd chromosome amounts to III −x=5.5% of the mitotic complement.

It is reasonable to identify the replicating 5.5% with the left arm and the 15.3% which are suppressed during polytenization with the right arm of the 3rd chromosome since the length of 3 L in mitotic follicle metaphases was 6.84% and of 3 R 16.28% (Fig. 3). This interpretation is supported by the morphology of the polytene 3rd chromosome of salivary glands (see p. 33).

6. Underreplication of the 3rd Chromosome

Onset of Underreplication. On the assumption that underreplication is the consequence of a single event which selectively interferes with the chromosomal endoreplication at an early larval stage and given that the non polytenizing part of the 3rd chromosome comprises 15.3% of the mitotic complement, it is possible to follow its endoreplication and relative decrease by means of a numerical simulation. The curves *A, B, C, G* in Figure 9a illustrate the assumptions that underreplication occurs before polytenization starts, or during

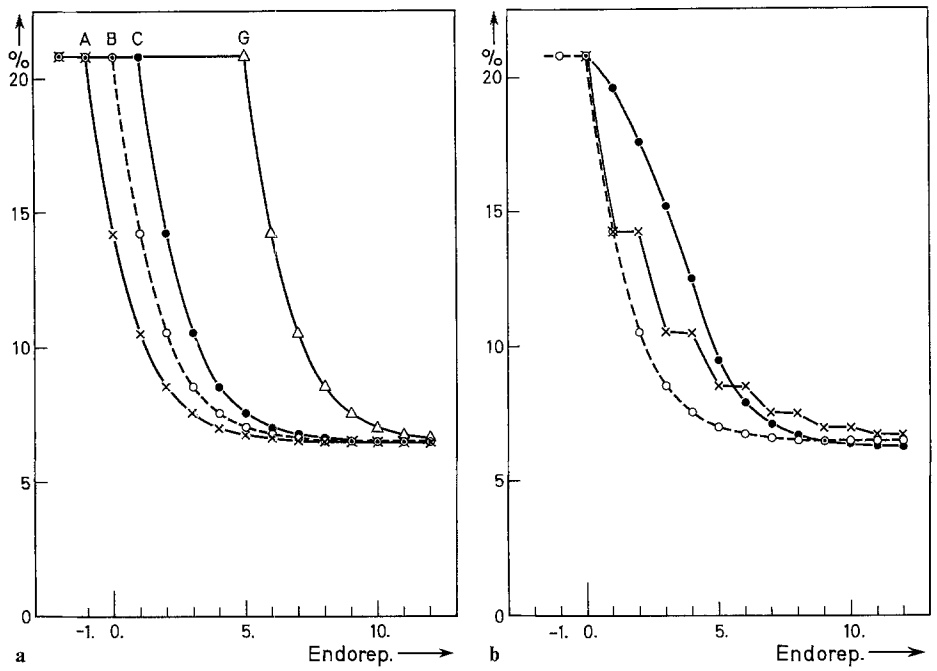

Fig. 9a and b. Numerical simulation of the relative decrease of the 3rd chromosome from 20.8% to 6.5% of the complement during polytenization. **a** Underreplication was taken as a single event in various cell cycles: during the first, second, sixth endoreplication, respectively (*B, C, G*). Hypothesis (*G*) does not fit the extinctions of 3rd chromosomes from 7th–8th endoreplication. Imputation (*A*) simulates a selective replication already in the cycle before polytenization starts (in time with differentiation). This idea was rejected because of major molecular difficulties. **b** Single event model (*A*: o---o, identical with *B* in **a**) compared to two other systems of selective polytenization: *B*: ×——× factor 0.5 slow replication, *C*: ●——● progressive underreplication which successively excludes 5 fractions (comp. Discussion, p. 47). All simulations are based on an underreplicated DNA fraction which represents 15.3% of the mitotic genome

the first, second or sixth endoreplicative cycle. Possibility *G* cannot accommodate the finding of a constant portion of 6.5% in the highly polytene complement. Hence the underreplicative event must occur before the 5th step of polytenization.

An exact definition of the time of underreplication is difficult insofar as the animals were not bred in the laboratory and therefore defined larval instars with very low DNA content in salivary gland nuclei were not available. One set of Feulgen measurements was performed on a salivary gland from a larva which was at the early 3rd instar. Five of the largest nuclei were measured. The 3rd chromosome possesses $6.6 \pm 0.3\%$ of the complement on average. A hen erythrocyte DNA standard showed that these 5 *Prodiamesa* nuclei were probably in the S-phase of the 8th endoreplication. This result suggests that possibilities *E* and *F* of Figure 9a, i.e. the 4th and 5th cycle, can also be ruled out.

In a somewhat smaller nucleus on the same slide, assigned to the S-phase of the 7th replicative step, the 3rd chromosome could not be morphologically distinguished.

Heteropyknosis. Attention has already been drawn to the fact that the 3rd polytene chromosome does not reveal segmentation in two arms. It stands in the same proportion to the other giant chromosomes as the left arm of a 3rd metaphase element to the metaphasic chromosomes 1 and 2. In other words: The 3rd giant chromosome mainly corresponds to the left arm of its original mitotic form. If there are still some chromatids of its right arm in the polytene 3rd, they should be found in the heterochromatic knob seen on the top of the cone (Fig. 7). Whether this knob is identical with the underreplicated right arm of the 3rd was checked by photometry. A sample of 11 duplicate readings showed that the heterochromatic clot represents $9.0 \pm 0.8\%$ of the 3rd giant chromosome; this is equivalent to 0.6% of the polytene complement.

On the basis of the assumption that polytenization ceases at the first endoreplication (*B* in Fig. 9a) the share of the right arm in the whole polytene 3rd should decrease between the 7th and 12th endoreplications as follows:

$$2.13\% - 1.07\% - 0.54\% - 0.27\% - 0.14\% - 0.07\%.$$

These values are much lower than the 9% detected for the heterochromatic knob, and are not greatly altered, if we suppose that underreplication happens one cycle later.

The unexpected result that the heterochromatic clot of the polytene 3rd chromosome contains more heterochromatin than the underreplicated right arm is supported by another observation: Early polytene 3rd chromosomes do not possess particularly large clots, and the DNA rich 3rd chromosomes do not show relatively small clots.

Exceptional DNA Contents. The 3rd chromosomes in position 2 and 11 of Table 2 are significant extremes: Relative DNA contents of 3.4% and 9.1% clearly depart from the mean of 6.5%. One may inquire whether these deviations are present in all nuclei of the corresponding glands. Measurements of as many 3rd chromosomes as possible were taken from the slides *EG 8* and *EG 4*, using *EG 3, 5, 10* as references. The extinction values of these 3rds were projected onto the distal portion of the left arm of the 1st chromosome (1 L) in the same nucleus. The results are combined in Figure 8. Data from reference slides *EG 3, 5, 10* demonstrate that the standard segment equivalent to 6.5% complemental portion is delimited by a double complex of discs (DC).

The salivary gland on slide *EG 4* in which an over-sized 3rd chromosome was found (9.1% of the complement) consists of 34 cells with highly polytene chromosomes; 20 were measured, 16 of which exhibit DNA values similar to the 3rd chromosomes of references *EG 3, 5, 10*. Three 3rd chromosomes had DNA values intermediate between normal and high.

Out of altogether 36 cell nuclei, 14 3rd chromosome equivalents were determined on slide *EG 8*; all of them mapped in a position representing a complemental portion of 3.4% (CC in Fig. 8).

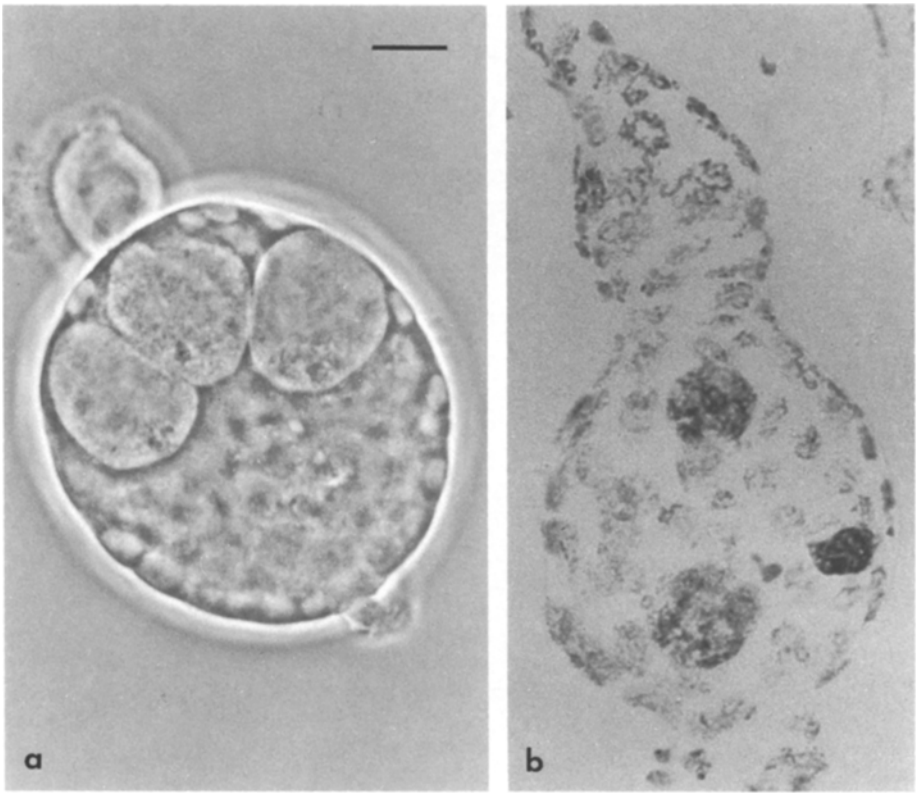

Fig. 10a and b. Pupal ovarioles each with two follicles. **a** Microdissected ovariole. The large primary follicle shows 3 endoreplicated nurse cell nuclei surrounded by nuclei of the envelope. Carmine-orcein acetic acid. **b** Squashed ovariole. The large follicle possesses two isophasic trophocyte nuclei and a third more condensed one. The trophocyte nuclei of the smaller follicle exhibit polyneme structure. Feulgen staining conceals the oocyte. Bar = 10 μm

The single 3rd chromosome of *EG 4* with 9.1% and all 3rds of *EG 8* with about 3.4% of their complements may be considered as instances of chromosomal aneuploidy, in other words: a trisomic origin in the one case and a monosomic in the others, all deriving from non-disjunction. In the case of *EG 8* (3.4%) it is possible that non-disjunction occurred in early cleavage mitoses or even in the meiosis.

D. Chromosomes in Nurse Cell Nuclei

The 3, sometimes 7 trophocyte nuclei of an ovariole (Fig. 10) show two kinds of endomitoses; one follows on the other during follicle development. Both exhibit cyclic alterations in chromatin condensation within a permanent nuclear membrane. The first kind of endomitosis is characterized by *polyneme* chromo-

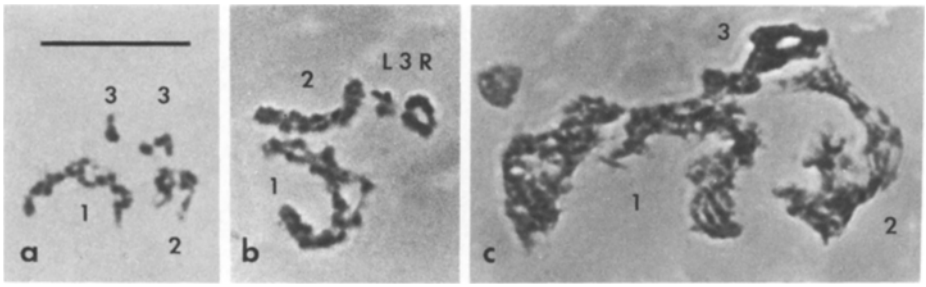

Fig. 11a–c. Endometaphases of nurse cell nuclei with increasing DNA content: **a** 8 C, **b** 16 C, **c** 64 C. The pairs of 3rd chromosomes are segmented in left and heterochromatic right arms. Feulgen staining. Bar = 10 μm

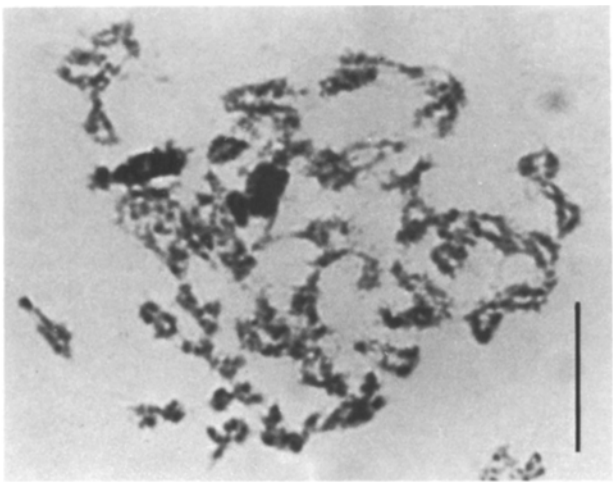

Fig. 12. Autopolyploid endometaphase of a highly endoreplicated nurse cell nucleus (second endomitotic stage). The 3rd chromosomes are heterochromatic and obviously still polyneme. Feulgen. Bar = 10 μm

somes. The endometaphases reveal three chromosomes somewhat longer than normal mitotic elements, but much shorter than genuine polytene chromosomes (Fig. 11). Since there are scarcely arguments for the multistrandedness of normal mitotic chromosomes (Beermann, 1961; Callan, 1972), the term polynemy can be applied to multifibered kinetic units of endomitoses. In *Prodiamesa* pupae these polyneme metaphases belong to the secondary follicles of ovarioles (Fig. 10b, upper part); they do not have a banding pattern and disintegrate at a DNA content around 64 C. After this the nuclei of trophocytes contain more than three chromosomes. The nurse cells of the advanced main follicles show such morphologically *polyploid* nuclei in the pupa (Fig. 12).

Table 3. Extinction values of polyneme chromosomes in endometaphases from Feulgen stained trophocytes. Double readings with analog UMSP; 0.5 µm diaphragm. Calculated data: *. Slides of the same Feulgen series as in Table 1. The endometaphases of nuclei 1, 5 and 14 were presented in Fig. 11a–c

Nucleus	1st chromosome		2nd chromosome		3rd chromosome		3 R		3 L		Sum of chromosomes AU	Total endometaphase AU
	AU	%	AU	%	AU	%	AU	%	AU	%		
1	31.80±0.20	49.92	18.10±0.10	28.42	13.80*	21.66	–	–	–	–	–	63.70±0.10
2	35.80±0.80	54.83	15.70*	24.04	13.80±0.70	21.13	–	–	–	–	–	65.30±2.10
3	68.25*	54.21	36.20±0.50	28.75	21.45*	17.04	15.40±0.42	12.23	6.05±0.05	4.81	–	125.90±1.40
4	66.50*	51.79	35.50±0.50	27.65	26.40*	20.56	20.30±0.10	15.81	6.10±0.00	4.75	–	128.40±4.20
5	68.90±0.50	51.88	37.20±0.60	28.01	26.70±0.10	20.11	21.95±0.25	16.53	6.55±0.15	4.93	132.80*	130.00±0.00
6	69.40±1.90	52.49	35.65±1.15	26.97	27.15±0.05	20.54	20.75*	15.70	6.40±0.30	4.84	132.20*	131.50±1.40
7	66.55*	50.49	38.50±0.70	29.21	26.75±0.25	20.30	–	–	–	–	–	131.80±0.80
8	67.75±2.05	52.93	34.85±0.45	27.23	25.40±1.40	19.84	19.53*	15.26	5.87±0.07	4.59	128.00*	132.75±0.75
9	74.30±0.30	51.37	39.10±0.10	27.03	31.25±0.35	21.60	24.20*	16.73	7.05±0.15	4.87	144.65*	141.30±0.90
10	69.85±1.85	48.92	45.00*	31.51	27.95±0.15	19.57	–	–	–	–	–	142.80±5.80
11	71.30±0.20	49.11	43.10*	29.68	30.80±0.80	21.21	–	–	–	–	–	145.20±3.40
12	71.50*	48.91	41.20±1.00	28.18	33.50±0.80	22.91	–	–	–	–	–	146.20±2.60
13	74.30*	50.61	42.90±1.30	29.22	29.60±0.10	20.17	–	–	–	–	–	146.80±1.80
14	305.00*	51.90	159.60±1.20	27.16	123.10±4.70	20.94	–	–	–	–	–	587.70±6.50
Mean of complemental portion		51.38		28.08		20.54		15.38		4.80		
Standard error of mean		0.50		0.46		0.36		0.67		0.05		
Coefficient of variation		0.04		0.06		0.06		0.16		0.02		

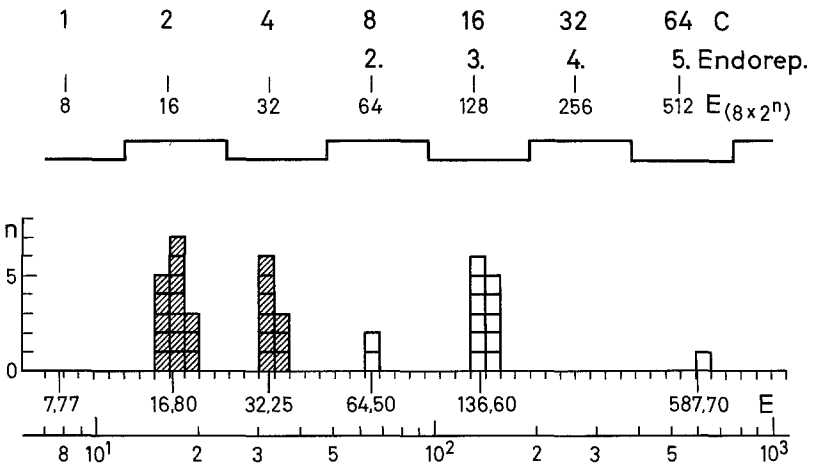

Fig. 13. Calibration histogram of trophocyte endometaphases (comp. Table 3). As Feulgen DNA standards 30 sperms ($\bar{x}_1 = 7.77$ AU; distribution not plotted), 15 G 1-nuclei (hatched; $\bar{x}_2 = 16.80$ AU) and 9 mitotic metaphases (hatched; $\bar{x}_3 = 32.25$ AU) were used. An overall mean of the standards provides the 1 C basis with 8 AU, thus the endometaphases (blank squares) belong to the 2nd, 3rd and 5th endoreplicative levels

The polyneme chromosomes of young nurse cells are accesible to microphotometry. Table 3 lists the data which are arranged in order of increasing content of Feulgen DNA. It is obvious that three classes were registered; between the two higher levels of total extinction one class is missing. The endometaphases were calibrated in Figure 13; using data from sperms, follicle nuclei of G 1-phase and of metaphases a hypothetical extinction of 8 AU for the 1 C level was obtained. Therefore, two trophocyte endometaphases are 8 C, eleven have a 16 C content, and only one has reached 64 C. Endometaphases with a 32 C content were not found.

At all these levels the relative proportions of the polyneme chromosomes are on a par with those of normal mitotic metaphases: 51.4% (1st), 28.1% (2nd), 20.5% (3rd).

The values clearly demonstrate that, in contrast to salivary gland nuclei, no underreplication of the 3rd chromosome takes place in trophocyte nuclei. The left arm of the polyneme 3rd chromosome comprises 4.8% and the heterochromatic right arm 15.4% of the endomitotic set. These photometric data are in good agreement with the relative lengths of the 3rd chromosome from normal mitoses (comp. Fig. 3).

Together with the arguments presented on p. 38 these considerations warrant the identification of the non polytenizing portion of the 3rd chromosome with its right arm.

IV. Discussion

A. Cytology of Underreplication

The phenomenon of underreplication was quantitated by microphotometry (Rudkin, 1965, 1969; Berendes and Keyl, 1967; Mulder et al., 1968); DNA

sequences subject to underreplication were also detected as satellites in density gradients or characterized by reannealing experiments (Rae, 1970, 1972; Gall et al., 1971; Ammermann et al., 1974; Moritz and Roth, 1976) and their distribution in chromosomes demonstrated by in situ hybridizations (Gall and Pardue, 1969; John et al., 1969; Hennig, 1973; Cordeiro-Stone and Lee, 1976; Steinemann, 1976).

The conditions in *Prodiamesa olivacea* are similar to the well documented cases in *Drosophila*. The relative size, as measured by Feulgen DNA content of the 3rd chromosome decreases from 20.8% to 6.5% during polytenizing endoreplications. A numerical simulation showed that this is best explained by assuming that roughly one quarter of the mitotic chromosome takes part in polytenization (5.5% of the complement), while the remainder (15.3% of the complement) is excluded from DNA syntheses. In normal metaphases the length of the right arm of the 3rd comprises 15–16% of the complement, and 3 L came to 5.3% and 7.1% in two neuroblastic or to a mean of 6.8% in eight follicle metaphases.

The agreement between the measured and calculated data as well as the peculiar morphology of the polytene 3rd chromosome give strong support to the idea that underreplication is essentially limited to its right arm. One would, however, obtain similar relative values if the two large chromosomes were also involved in a balanced underreplication of amounts of DNA which are proportional to their metaphase values and do not exceed 10% of each chromosome. Such a situation is highly unlikely for the following reasons:

The 3rd chromosome in nurse cells does endo- but not underreplicate. The right arm of this chromosome is heterochromatinized and contains 15.4% of the total Feulgen DNA. The euchromatic left arm of the 3rd in these trophocytes, with 4.8% of the complement, corresponds to the banded part of this chromosome in salivary glands. In salivary glands, this euchromatin corresponds to 5.9% of the total complement. These two values are in agreement, when one considers that the salivary gland nuclei largely lack the sequences of 3 R.

The heterochromatic clot represents 9% of the polytene 3rd chromosome. If 3 R were to remain diploid, this arm would comprise after 10 endoreplications a DNA fraction of only 0.27% of the 3rd chromosome or of 0.018% of the complement; after 12 endoreplications 0.07% or 0.004%, respectively. Hence this dense clot must consist of more DNA than the underreplicated 3 R.

Furthermore, autoradiograms show this heterochromatic knob to be a locus of intensive ^{3}H-thymidine incorporation (Fig. 7d). Therefore, this structure presumably consists of two components: the underreplicated right arm of the 3rd chromosome, α-heterochromatin after Heitz (1934), which is concealed by another late replicating material harmonizing with the definition of β-heterochromatin, despite its high condensation.

Such long- or late-replicating material is not restricted to the 3rd but was also found in the two other polytene chromosomes (Fig. 14), where it is more or less symmetrically arranged in two blocks adjacent to the kinetochore.

To summarize the findings on the structure of the 3rd chromosome it can be said that the left arm contains 4.8–5% of the complemental DNA in the mitotic state. It participates in all polytenizing endoreplications and has a typical banding pattern. The underreplicated right arm (α-heterochromatin) is concealed

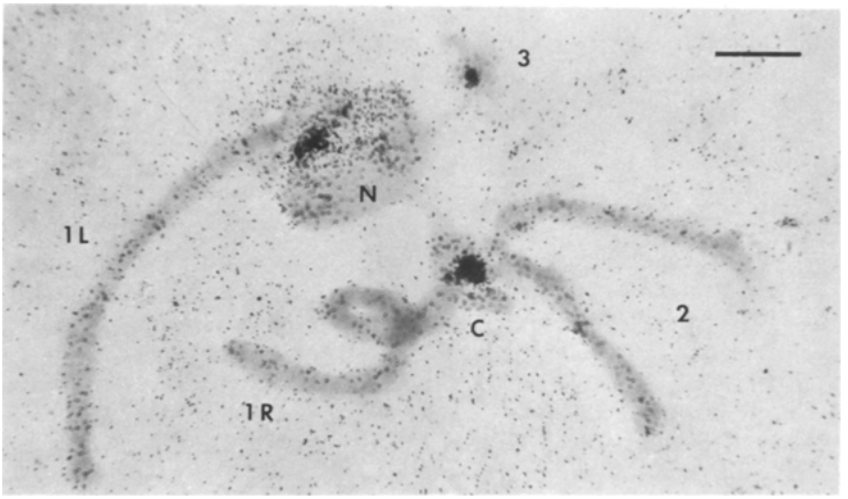

Fig. 14. Long- or late-replicating loci in salivary gland chromosomes. Silver grains mainly mark the chromocenter (*C*), heterochromatic material within the nucleolus (*N*) and the dense knob of the 3rd. Unstained ^{3}H-thymidine autoradiogram. Bar = 20 μm

in dense replicating material (β-heterochromatin) which is presumably symmetrically distributed around the kinetochore. The α- and β-heterochromatin together make up the heterochromatic knob which represents about 10% of the polytene 3rd. The β-heterochromatin was not identified in diploid or less endoreplicated nuclei as it constitutes only ca. 0.5% of these complements.

B. Time of Underreplication

In *Drosophila melanogaster* a partial replication stop can occur during the first endoreplications (Rudkin, 1965, 1969), although it is not certain that all nuclei respond at the same time or to the same extent.

For *Prodiamesa olivacea* it is possible to calculate the approximate time of underreplication as a single event from the proportions of the 3rd chromosome to the total DNA at particular levels of endoreplication. Figure 9a indicates that 6.6% relative DNA content of 3rd chromosomes at the 8th endoreplication (result from a larva at the early 3rd instar) requires that DNA synthesis in its right arm stops before the 4th endoreplication. A more exact determination is not possible since the 3rd is not amenable to measurement before the 7th endoreplication.

Perhaps it is more reasonable to assign underreplication to a *period* of time (endoreplication 1 to 3) than to regard it as a single event, for endoreplicated cells in brains and salivary gland anlagen of *Drosophila hydei* possess chromocenters with 2 C, with 4 C and some with 8 C content (Berendes and Keyl, 1967; Mulder et al., 1968). The uncoupled replication of heterochromatin and euchromatin suggests two largely independent mechanisms of regulation.

Figure 9b compares the single factor underreplication (*A*) with two variations, all starting with the first endoreplication. The first system (*B*) is based on the premise that the underreplicated DNA is alternately excluded from replication. This model is formally equivalent to "slow replication" (Rudkin, 1965) but it is not supported by the *Prodiamesa* data, because the 3rd chromosome would reach a constant 6.5% of the polytene set at a later stage than was found.

In contrast a progressive underreplication (*C*) is much more difficult to reject; this conception is based on several DNA fractions which are successively prevented from DNA synthesis. Even with the five fractions, assumed arbitrarily here, it is difficult to distinguish between the result of this simulation and that of a single event because no data are available for the area in which the curves diverge. Clearly a two-fraction model, similar to the two-stage chromosome elimination in *Sciara coprophila* (Metz, 1938) or chromatin diminution in *Cyclops furcifer* (Sigrid Beermann, 1966, 1977) causes the curve (*C*) to converge even more closely onto that of a single event.

Figure 15 shows that the extinction values of polytene 3rd chromosomes in *Prodiamesa* have a broad dispersion about 7 times larger than a mean relative error of $1.9 \pm 0.2\%$, obtained after 31 repeated readings of single 3rd chromosomes. This marked scatter raises the possibility that DNA fractions are excluded

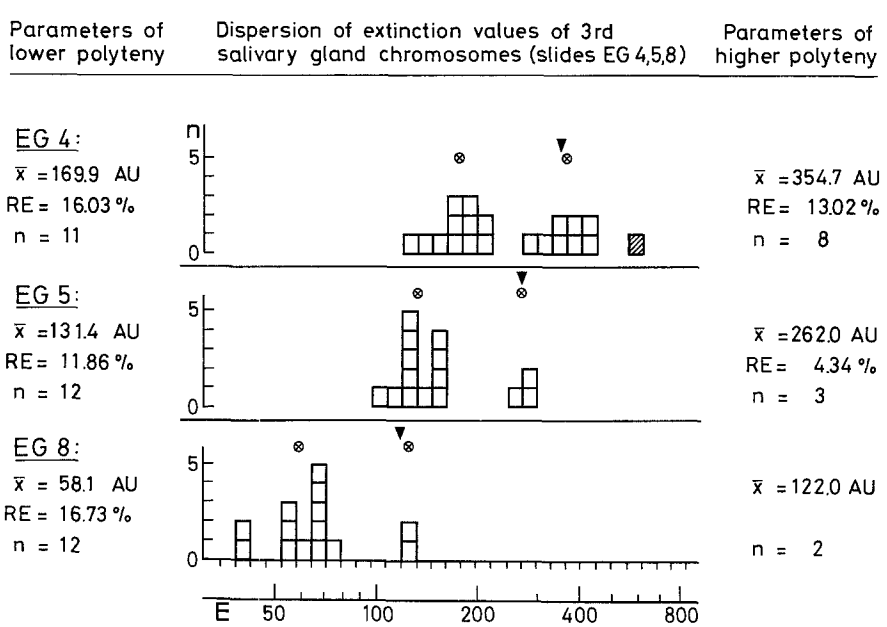

Fig. 15. Scatter of DNA contents of 3rd salivary gland chromosomes from slides *EG 4, 5, 8*. The salivary gland of slide *EG 4* contains 34 cells, 20 were measured. The hatched square is the extinction of the 3rd with the extreme complemental portion of 9.1%; therefore it was not sampled within the higher polyteny level. *EG 5:* 30 cells, 15 nuclei measured. *EG 8:* 36 cells, 14 nuclei measured. All 3rd chromosomes of slide *EG 8* possess an exceptional relative DNA content of 3.4% (comp. Fig. 8). $\bar{x}$ Mean (= ⊗); twofold mean of lower polyteny: ▼. *RE* Relative error (= 100 × standard deviation: mean)

from DNA syntheses at random, i.e., underreplicated areas are not under rigid control (Keyl and Hägele, 1966). The observed pattern of scattered underreplication in the Y-chromosome of *Phryne cincta* can best be accounted for by assuming that the proportion of replicating DNA varies in different cells (Sokoloff and Zacharias, 1977). After the heterochromatin-definition of Heitz (1934) this would imply a conversion of β-heterochromatin to α. Another idea allows underreplicated chromosomes to vary among one another by differing rates of DNA synthesis. This is an extrapolation from the observation that the efficiency of a DNA polymerase increases in HeLa cells during S-phase (Spadari and Weisbach, 1974).

It is conceivable that the phenomenon of underreplication is due to enzymatic removal of DNA (Berendes and Keyl, 1967). Such enzymatic restriction would imply the total elimination of the sequences in question. That this does not occur is clearly demonstrated by in situ hybridizations of underreplicated satellite sequences in polytene chromosomes in *Drosophila* (Gall, 1971; Steinemann, 1976).

It stands to reason that underreplication is first detectable at the 4 C level. It is of interest to ask whether such a relative selection of DNA sequences is confined to polytene structures. The extremely polyploid cells of the silk glands in *Bombyx mori* do not lose repetitive sequences (Gage, 1974), likewise several *Dermestes* species show in testis wall and fat body geometric progressions of DNA beginning with 2 C values (Fox, 1969, 1970a).

In *Schistocerca gregaria* and *Locusta migratoria,* however, the somatic tissues do not carry out complete endoreplications (Fox, 1970b). Conventionally polyploid cells which are underreplicated were also verified in the gall midge *Heteropeza pygmea* which lacks highly repetitive sequences of the kinetochoric region in endoreplicated fat body cells (Kunz and Eckhardt, 1974).

Prodiamesa underreplication is limited to nuclei with polytene chromosomes. The characteristic appearance of the polytene 3rd was not only detected in salivary glands but also in the Malpighian tubules and the hind gut, whereas the polyneme nuclei of trophocytes are fully replicated but 3 R is heteropycnotic and hence probably inactive. This fact is an indication that underreplication can be considered as a somatic event; it is in agreement with the postulate that heterochromatinization is a restrictive process like elimination in that both serve to silence DNA (Sager and Kitchin, 1975). This also implies that trophocytes are the last cells in ontogeny which differentiate away from the germ line and become quasi soma cells. Their somatic nature is confirmed by the underreplication of the heterochromatic X-chromosomes in nurse cells of *Musca domestica* (Bier, 1962) and of rDNA in *Drosophila hydei* and *D. virilis* (Renkawitz and Kunz, 1975; Renkawitz-Pohl and Kunz, 1975). This view is supported by the occurrence of polytene chromosomes in trophocytes (Stalker, 1954; Bier, 1960).

The function of DNA sequences which are later underreplicated is unknown. The few available data (Miklos and Nankivell, 1976; Geyer-Duszyńska, 1966; Bantock, 1970) would tend to assign a role in recombination or the production of fertility factors to these silenced sequences.

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