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General characteristics of the polytene chromosomes from ovarian pseudonurse cells of the *Drosophila melanogaster otu*^{11} and *fs(2)B* mutants

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Polytene chromosomes of good cytological quality from pseudonurse cells (PNCs) of *fs(2)B* and *otu*^{11} mutants were obtained, photomaps for *otu*^{11} mutants were constructed and the general characteristics of polytene chromosomes from salivary glands (SGs) and PNCs were compared. Three conditions were found to improve the cytological quality of PNC chromosomes: temperature below 18°C, a protein-rich medium and presence of the Y-chromosome. Detailed comparison of the chromosome banding pattern from SGs and PNCs has shown only minor differences between them. The frequency of asynapsis appeared to be 10 times higher for PNC chromosomes. Despite previous reports, features such as breaks and ectopic contacts turned out to be also typical for PNC chromosomes, but with remarkably lower frequencies.

Key words: banding pattern, chromosomal photomaps, heterochromatin, polytene pseudonurse cell chromosomes

Introduction

In the present study a new attempt was made to compare polytene chromosome activity and banding pattern in different tissues of *Drosophila*. Investigations of this kind have already been performed in other organisms such as *Anopheles stephensi* (Redfern 1981), *Calliphora erythrocephala* (Ribbert 1979) and *Melanagromyza obtusa* (Gupta & Singh 1983), and have aroused great interest. In *Drosophila melanogaster* polytene chromosomes are normally available for analysis in the larval stage only. Comparisons between SG chromosomes and chromosomes from fat body (Richards 1980), hindgut (Zhimulev *et al.* 1982), hindgut, midgut and the protoracic gland (Hochstrasser 1987), midgut of *Drosophila gibberosa* (Roberts 1988) and the protoracic gland from DTS-3 mutants of *Drosophila melanogaster* (Holden & Ashburner 1978) did not show great differences between chromosomes of larval tissues.

Isolation of female sterile mutations that cause the formation of polytene chromosomes in normally endopolyploid nurse cell nuclei (these mutant nurse cells are called pseudonurse cells, PNCs), such as *fs(2)B* and *otu*^{11} (King *et al.* 1978) provided the opportunity to study the polytene organization of germ-line cell chromosomes. It is now possible to compare chromosomes not only from different stages of metamorphosis but also from the different cell types of soma and germline.

Comparative analysis of SG and PNC chromosomes was first carried out by King *et al.* (1981). Later, Sinha *et al.* (1987) also showed remarkable similarity in these two cell types for *otu*^{1}/*otu*^{3} flies. Heino (1989, 1994) published detailed photomaps for X, 3L and 3R from PNCs of *otu*^{1} mutant and found minor banding differences only. As to general characteristics, neither constrictions or breaks nor ectopic pairing were found in PNC chromosomes.

The data reported so far have stimulated our interest in euchromatin–heterochromatin relationships in PNC chromosomes. To investigate these relationships a rather simple method for preparing a large number of chromosomes of good quality was needed. Having developed such a method it became possible to construct photomaps, as presented in this paper. A comparative analysis of the maps obtained and those of SG chromosomes revealed some discrepancies with the results published previously, suggesting more differences in chromosomal organization.

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Materials and methods

Stocks

Female sterile mutation *fs(2)B* with markers *cn* and *bw* was balanced with *CyO*. (For a detailed description of all used balancers see Lindsley & Zimm 1992.) The stock was maintained on standard medium at 17–18°C.

Mutation otu^{11} was marked with *y*, *w*, sn^3 and balanced with *FM3*. To produce flies of $\widehat{XXY}$ genotype, males with attached X and Y chromosomes were obtained, where X carried *y*, *w*, sn^3, otu^{11} and Y was marked with y^+ duplication of X-chromosome material. Flies were maintained on standard medium at 16°C.

Cytological procedures

The mutant ovaries were dissected on day 6–8 after eclosion in the case of females homozygous for *fs(2)B* or otu^{11} and on the fifth day for the case of *y w sn3* otu^{11}/ *y w* sn^3 otu^{11}/y^+ Y^L Y^S females. (It is interesting to note that the presence of Y chromosome accelerates the development of the ovaries.) The mutant ovaries from females anaesthetized with ether were dissected in Ringer solution (Ephrussi & Beadle 1936) and stained in acetoorcein for 1 h. Ovarian chambers containing PNC chromosomes after selection were squashed in 55% lactic acid.

For comparative analysis, acetoorcein-stained preparations of SG polytene chromosomes were made and also squashed in 55% lactic acid. All observations were made by phase-contrast microscopy.

Groups of 50 chromosomes were studied in all analyses. Regions of the PNC chromosome photomap were subdivided according to Bridges standard maps (Lindsley & Zimm 1992) and Lefevre (1976).

Results

General characteristics

As PNC chromosomes are very different in their morphology and level of polyteny (King *et al.* 1981), we used for investigation and statistical analysis only chromosomes similar in appearance to those from salivary glands. The frequency of obtaining such chromosomes was 5–10%. The general view of PNC chromosomes with clear-cut banding pattern can be seen in Figures 1 & 2. One of the main features of *fs(2)B* chromosomes is the incomplete association (secondary splitting) of the chromatids. Asynapsis can be seen both between two homologues and between bundles of threads inside each homologue (Figures 1 & 3). Sometimes we found complex reconjugation of parts of different homologues (Figure 3b). Such splitting of individual homologues is very rare in otu^{11} chromosomes, but asynpasis between homologues in PNC chromosomes of otu^{11} mutants is much more frequent than in SG chromosomes (Figures 2 & 4). The percentage of asynapsed chromosome 4 of otu^{11} is also very high and equal to 32% for XX and 16% for $\widehat{XXY}$.

At variance with the opinion that the absence of a chromocentre is due to the strong under-replication of heterochromatin (Dabbs & King 1980, Heino 1989), we found additional blocks of heterochromatin in PNC chromosomes both in *fs(2)B* and otu^{11} (brackets on Figures 1, 2 & 3a; see also Mal'ceva & Zhimulev 1993). Chromosomes, however, are very often not united in a chromocentre, in accordance with the observations of the above-mentioned authors.

In contrast to the reported complete absence of weak points in the chromosomes of germline cells (Heino 1989, 1994) we observed breakage at certain sites (see, for example, Figure 7). Figure 5 summarizes the data on the frequencies of weak points. They evidently occur at least at 45 sites, but their frequency is substantially lower in PNC polytene chromosomes than in those of salivary glands. Nevertheless, their frequencies are similar in certain regions, namely in 19E, 36D and 39DE. The effect of the Y chromosome on frequency of breaks is quite weak, which can be ascribed to the low temperature, which hinders chromosome breaking (see for discussion Zhimulev *et al.* 1989). Although much less frequent than in salivary gland cells, we were able to detect ectopic conjugation in PNC of otu^{11} (Table 1). Remarkably, the same regions of intercalary heterochromatin are involved in the ectopic contacts in both tissues. We have no quantitative data for the chromosomes of *fs(2)B* mutants.

Photomaps of PNC polytene chromosomes

Photomaps of PNC chromosomes were made for otu^{11} mutants only (Figures 6–10). Thorough comparison of the banding pattern of SG and PNC chromosomes have revealed good correlation except in regions marked by brackets on the maps and in distal pericentric regions, such as region 20C–F, region 41–42, which somewhat resembles a broom in PNC chromosomes (Figure 8), and section 80, which is often diffuse but sometimes with a banded pattern (Figure 2). In many cases the corresponding bands in SG chromosomes are split in PNC chromosomes (21C1–2, 22C, 23D1–2, 23E1–2, 30AB, 48F, 51AB, 55C, 57E, 61C, 65D, 66E, 71A and 93F regions).

Not very many large puffs have been found in PNC chromosomes. The main ones are: 3CD, 7E, 8C, 11B, 22F, 42AB, 47A, 79D. Puff 47A is very much like a Balbiani ring. This type of structure can be found very rarely in the *Drosophila* group (Scouras & Kastritsis 1984). Another giant puff developed from a series of bands could be seen sometimes in the telomeric region 61AB in PNCs (data not shown).

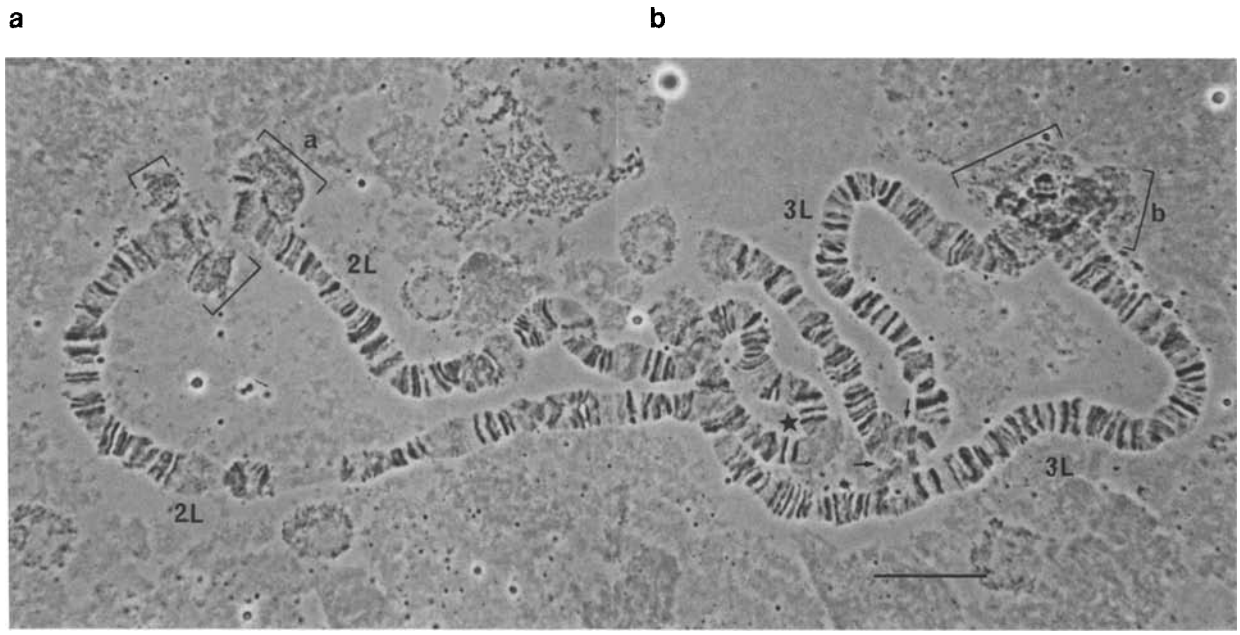

Figure 1. General view of the 2L and 3L chromosomes in the nucleus of nurse cell of a female homozygous for *fs(2)B*. Each chromosome limb is represented by two non-conjugated homologues (except the 2L telomere, marked by an asterisk and the region marked by arrows, where bundles of chromatids of the two 3L homologues conjugate in criss-cross manner). Parentheses indicate the chromocentral regions of 2L (**a**) and 3L (**b**) chromosomes. Bar = 10 μm.

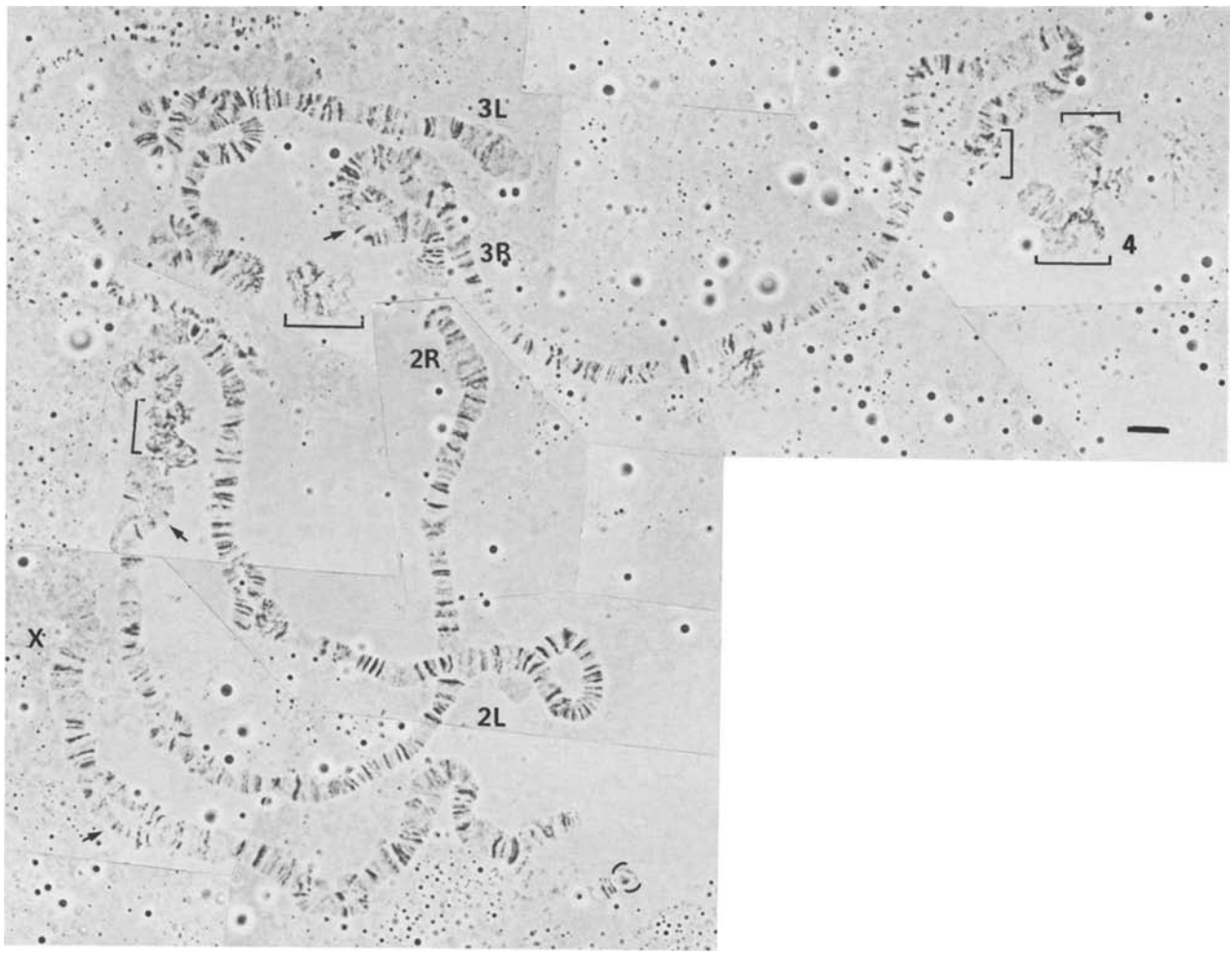

Figure 2. General view of the nurse cell nucleus of *ywsn*^{3}*otu*^{11}/*ywsn*^{3}*otu*^{11},*y*$^{+}$ Y^{L}Y^{S}. The square brackets indicate chromocentral regions of chromosomes, the round brackets point to what is presumably a Y chromosome from the *ywsn*^{3}*otu*^{11},*y*$^{+}$ Y^{L}Y^{S} homologue. Arrows point to non-conjugated regions. Bar = 10 μm.

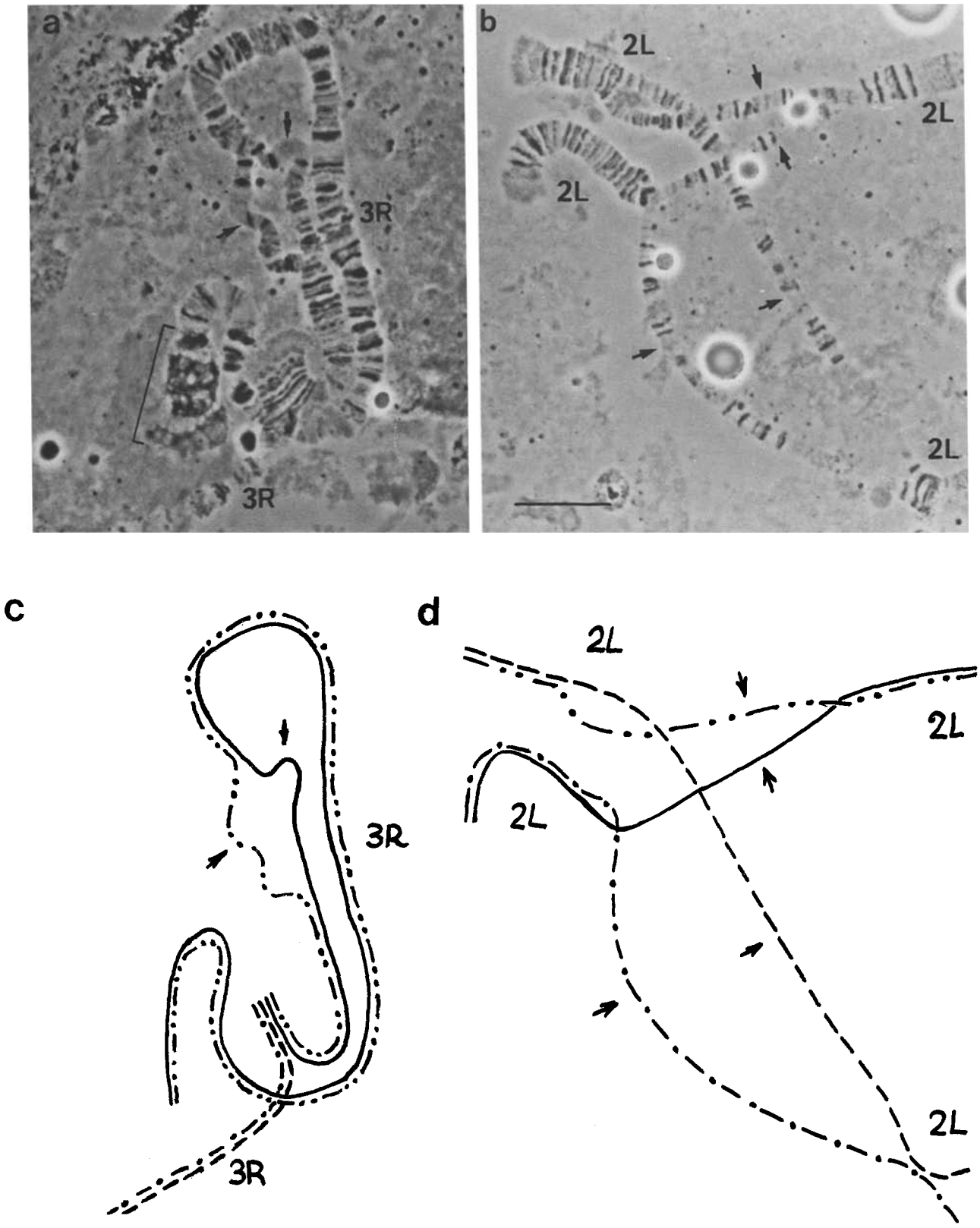

Figure 3. Examples of the complex asynapsis in 3R (**a**) and 2L (**b**) (shown schematically in **c** and **d** respectively) chromosomes from *fs(2)B* mutants. The bracket marks the pericentric heterochromatin of the third chromosome; arrows show complex asynapsis in 2L (**b**) and asynapsis in one of 3R homologues (**a**). Bar = 10 μm.

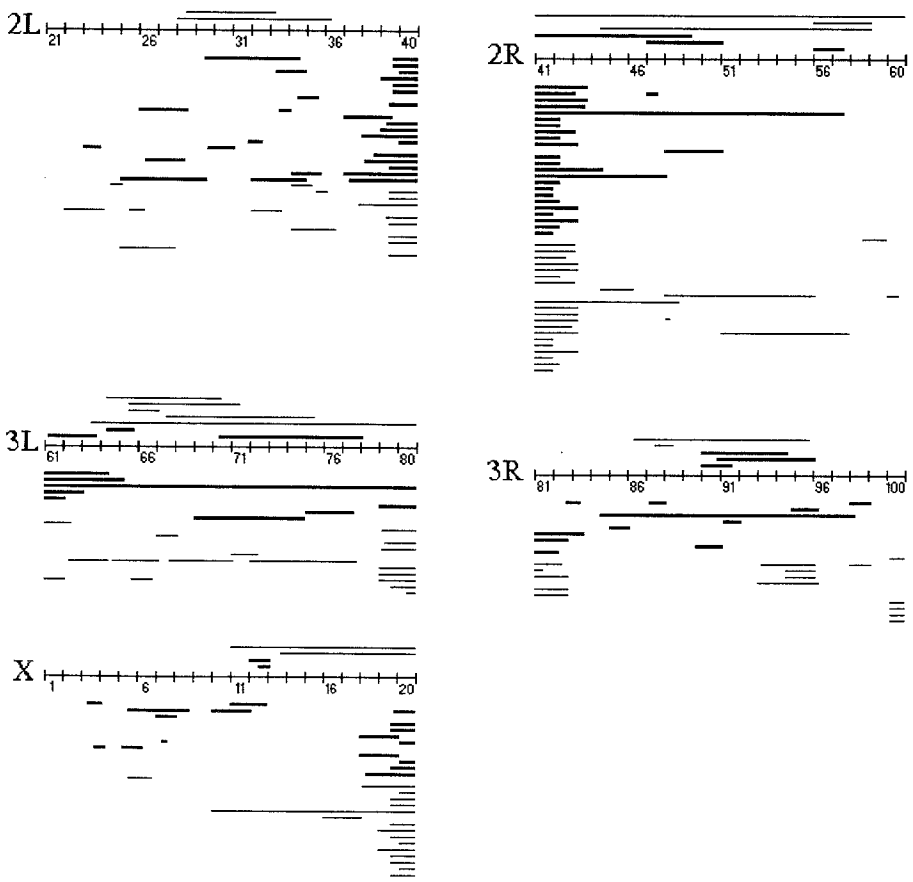

Figure 4. Asynapsis in polytene chromosomes of SGs (above the axis) and PNCs (below the axis) of *otu*[11] mutants. Numerical sections of polytene chromosomes are shown on the horizontal axis. The length and number of lines at the same level above and below the axes reflect the length of non-conjugated regions of the individual chromosome. Thick lines represent data on XX larvae and thin lines data on XX̂Y larvae.

Discussion

It is well known that maintenance conditions may influence the cytological quality of polytene chromosomes (for review, see Zhimulev 1992). Conditions such as low temperature and protein-rich food raised the frequency of obtaining PNC chromosomes from *fs(2)B* and *otu*[11] mutants with a similar cytological structure to SG polytene chromosomes.

Freed & Schultz (1956) have shown that adding of the Y chromosome causes an increase in the DNA content of nurse cell nuclei. The influence of the Y chromosome on the structure of the PNC polytene chromosomes is striking. In our experiments with XX̂Y females it was found that the frequency of ovaries showing well-banded chromosomes is markedly increased with improvement in the pericentric heterochromatin structure (Mal'ceva & Zhimulev 1993). It was interesting to see what Y chromosome looks like in PNCs. Only one structure can be regarded as a Y chromosome (Figure 2, round brackets), but this proposal must be verified by hybridization *in situ*.

Asynapsis is one of the main characteristics of chromosomal behaviour. For SG chromosomes it was found that frequency of asynapsis is different in different chromosomal regions. It is higher for the proximal part of the X and middle regions of the arms of the autosomes (Belyaeva 1973). As to PNC chromosomes, regions 19–20 of the X chromosome and 39D–42BC of chromosome 2 are the main locations of asynapsis (Figure 4). One of the features of PNC chromosomes is their ability to split on homologues and even on polytene threads. While the latter occurred very rarely in *otu*[11] mutant, it is rather frequent in *fs(2)B* (Figures 1 & 3), perhaps reflecting a greater similarity to wild type, in which chromosome threads are not aligned in register at all. This assumption is supported by the ability of *fs(2)B* homozygous females to produce, albeit extremely rarely, viable offspring when raised at low temperature (17°C; H. Gyurkovics, unpublished observation). Similar types of asynapsis, in which homologues are split into four polytene threads, have been observed in *Anopheles atroparvus* (Stegniy 1979). Splitting of chromosome homologues into two, three, four, five and even six bundles has been found in *Cerodontha dorsalis* (Agromyzidae) (Stalker 1954). The difference in the manifestation and its irregularity make the functional meaning of such splitting unclear.

Ribbert (1979) in *Calliphora* and Heino (1989, 1994) in *Drosophila* found no breaks, constrictions or ectopic pairing in the nurse cell polytene chromosomes. They postulated that this was due to the peculiarities in the organization of germ-line cell chromosomes. The data obtained in the present experiments have shown that

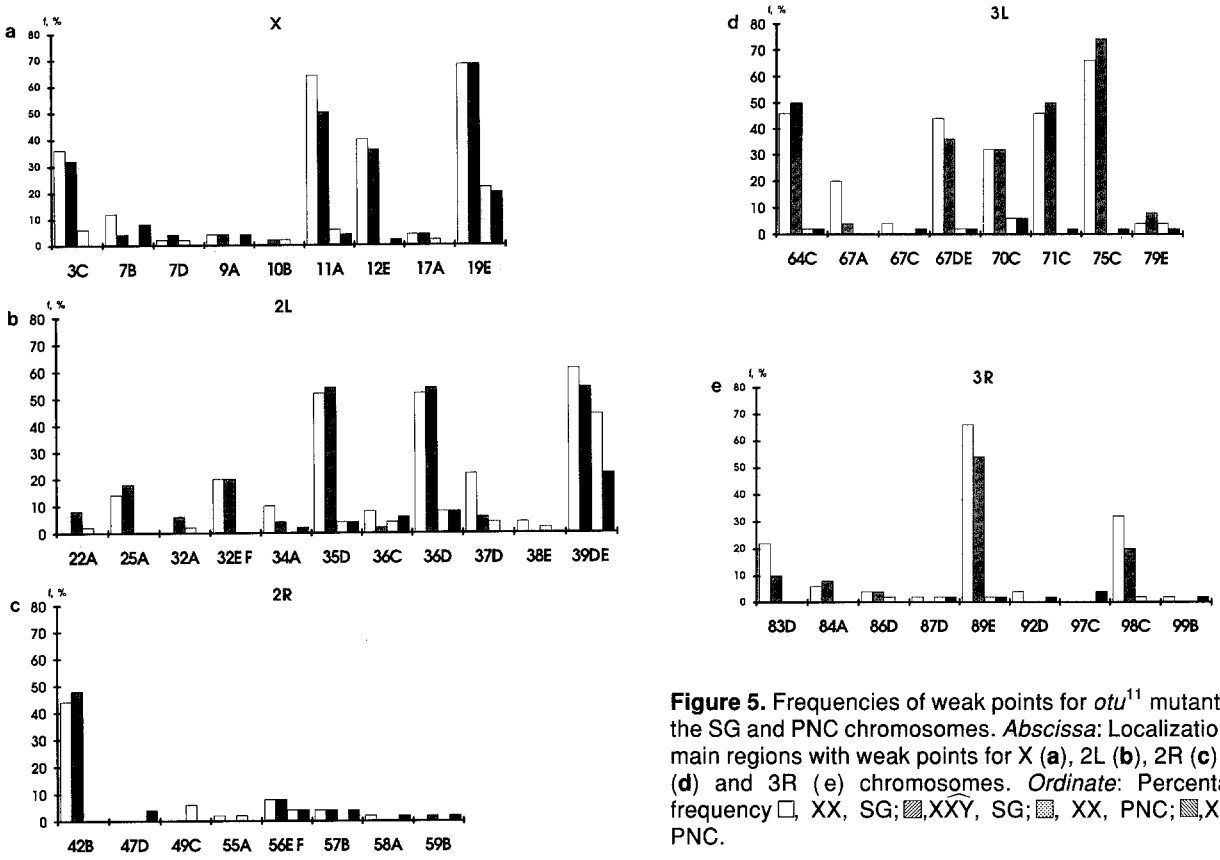

Figure 5. Frequencies of weak points for *otu*[11] mutants in the SG and PNC chromosomes. *Abscissa*: Localization of main regions with weak points for X (**a**), 2L (**b**), 2R (**c**), 3L (**d**) and 3R (e) chromosomes. *Ordinate*: Percentage frequency □, XX, SG; ▨,XX̂Y, SG; ▩, XX, PNC; ▧,XX̂Y, PNC.

PNCs do exhibit these features, but to a lesser extent than SG polytene chromosomes (Figures 4 & 5).

The question of heterochromatin organization arises in connection with these chromosomes. In particular, it is of interest to determine the nature of the classical pericentric heterochromatin for the X chromosome, since it exhibits banded organization but little thread-like net at the base of the chromosome. At the same time bands in 20A–C display the usual heterochromatic behaviour, in contrast to 20D–F. It would be of benefit to determine the location of the centromeric region of this chromosome.

We would like to point out that most of the unusual features of PNC chromosomes discussed so far, such as infrequency of constrictions, breaks or ectopic pairing, the presence of additional facultative heterochromatin and the apparent lack of chromocentre, can be explained by the simple assumption that some chromosomal proteins, in particular heterochromatic proteins, may be reduced in or missing from nurse cell and PNC nuclei. This also could lead to a reduction in heterochromatin under-replication (see Mal'ceva & Zhimulev 1993).

Considering the complex function of nurse cells, the relative scarcity of big puffs in PNC chromosomes is somewhat surprising. Our observations show, however, that numerous small puffs arise during the life cycle of these chromosomes and several big puffs, such as 3CD and 8C, can also be found (see Figure 6a). The structure of the 47A puff resembles the Balbiani ring, a phenomenon very rare in *Drosophila* species. Until now such structures have been found in SG polytene chromosomes of *D. auraria* and a few close relatives (Kastritsis & Grossfield 1971) and in a species of the *D. virilis* group (W. Beermann in Scouras & Kastritsis 1984).

The problem of the constancy of banding patterns of polytene chromosomes from different tissues appears to be simple and complex at the same time. While it was found to be difficult to detect homology between SG and nurse cell polytene chromosomes from *Anopheles superpictus* and *Anopheles stephensi* (Coluzzi *et al.* 1970) and between polytene chromosomes from trichogen cells and ovarian nurse cells from *Calliphora erythrocephala* (Ribbert 1979), Redfern (1981) for *Anopheles stephensi* and Heino (1989, 1994) for *Drosophila melanogaster* have shown striking similarity of banding pattern in SG and PNC cells.

The results obtained in the present study show good correlation of SG and PNC chromosome banding patterns for large and middle-sized bands, easily detectable under the light microscope for the majority of chromosomal regions. Exceptions were found for five

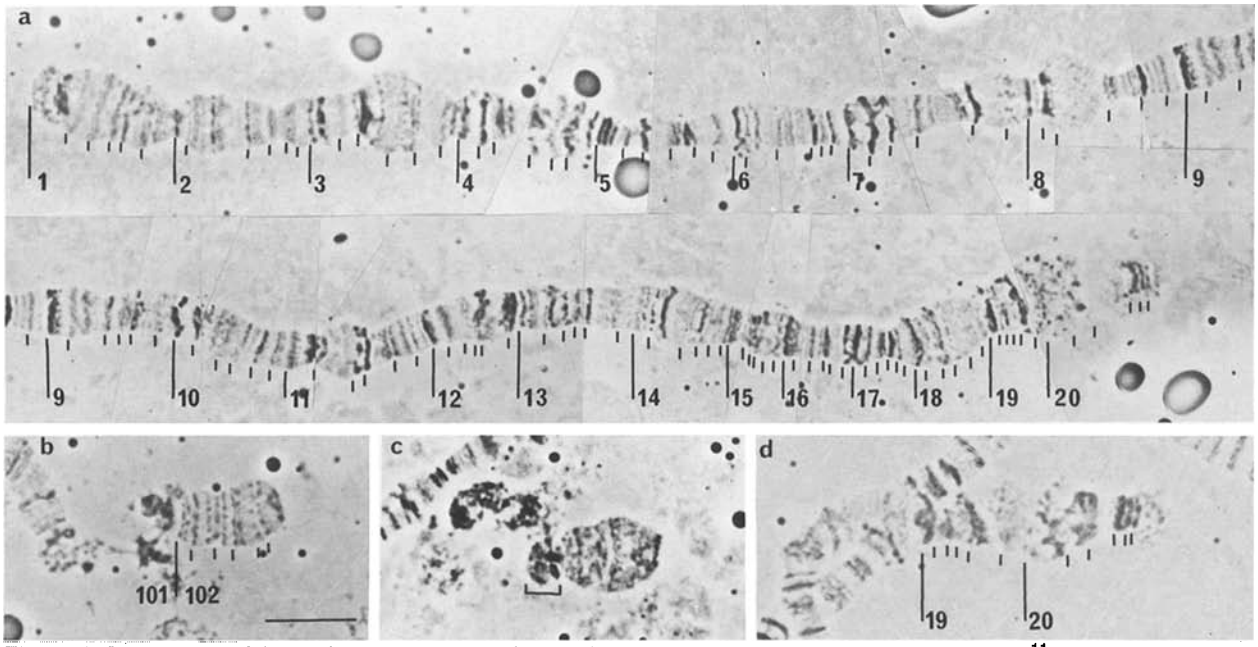

Figure 6. Photomaps of the X chromosome (**a** & **d**) and chromosome 4 (**b** & **c**) from a PNC of an *otu*[11] mutant. The bracket marks granular material to the left of the 102A region, which often could be seen in chromosome 4. Bar = 10 μm.

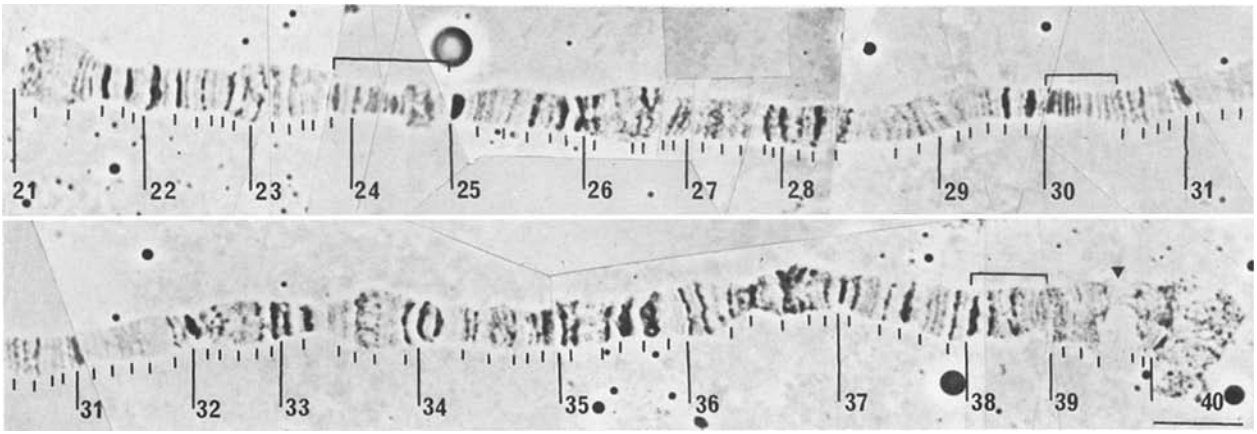

Figure 7. Photomap of the 2L chromosome from a PNC of an *otu*[11] mutant. Brackets denote regions where no similarity with the SG polytene chromosomes was found. Arrowhead marks weak point in 39DE region. Bar = 10 μm.

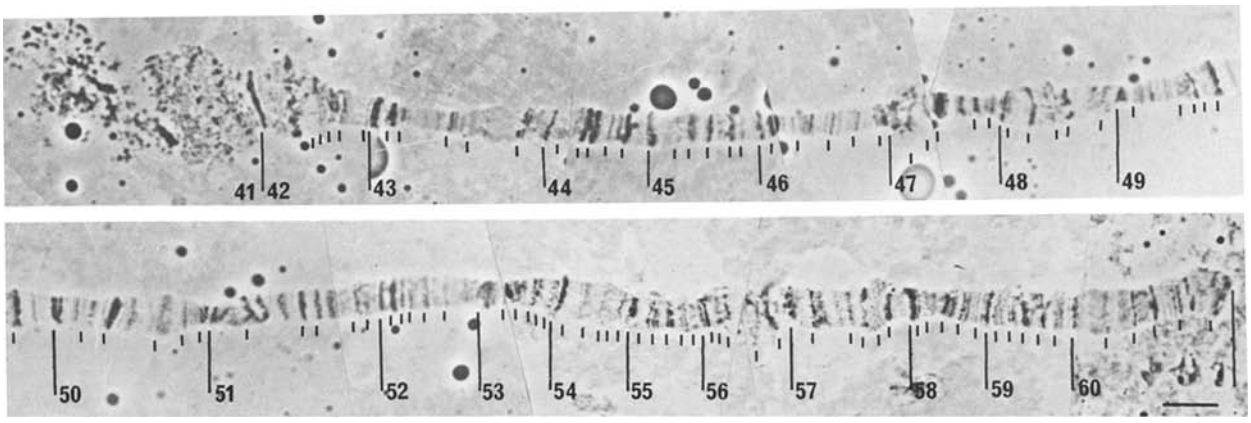

Figure 8. Photomap of the 2R chromosome from a PNC of an *otu*[11] mutant. Bar = 10 μm.

Table 1. Ectopic pairing in salivary gland (SG) and pseudonurse cell (PNC) chromosomes from *otu*[11] mutants (chr, chromocenter)

Genotype tissue	Pairs of regions with ectopic contacts between them
X chromosome	
XX, SG	4E–5D; 4D–7B; 11C–12EF; 12D–13C; 17A–19E; 18C–19A; 11A–12F;12F–19E; 11A–12D; 2E–3C; 2B–4D; 7D–8E; 7D–9A; 11A–12A; 12E–16A; 11A–12EF; 8E–10B; 10B–12E; 9A–9E; 18B–19A
$X\widehat{XY}$, SG	4D–7C; 3C–4D; 4E–6A; 10A–11E; 19A–20A; 4E–11A; 18C–19E; 4D–7B; 11A–19E
XX, PNC	9A–11A; 10A–12E; 2C–3AB; 11D–12D
$X\widehat{XY}$, PNC	
2L chromosome	
XX, SG	34A–34EF; 35A–39D; 39D–40E; 33B–86C; 36C–84F; 39A–39D; 25E–26D; 38E–39D; 39D–40A; 36D–37D; 37D–40A; 33EF–35D; 35D–36D; 32E–36D; 35CD–36D; 37A–39D; 34A–35D; 37D–40A; 36C–37D; 34A–35D; 25A–26C; 25D–26D; 35D–39D; 34D–35A; 38A–39A; 37D–38A; 38A–39D; 32E–33D
$X\widehat{XY}$, SG	25C–27F; 35A–36D; 37D–39D; 38D–40D; 32F–36C; 35CD–36D; 37D–chr; 22A–28D; 37B–38A; 39D–40D; 35D–36D; 33CD–34A; 32F–36D; 30A–31A; 35D–36D; 35CD–36D; 35CD–36CD; 32F–35D; 36CD–39DE; 32F–35D; 32F–38A; 35D–36D
XX, PNC	26C–28D
$X\widehat{XY}$, PNC	
2R chromosome	
XX, SG	58A–59A; 56E–4; 40EF–42B; 56A–57A; 59A–60C; 42B–43E; 57D–60A; 56F–59A; 41D–43A; 42D–44D
$X\widehat{XY}$, SG	57B–58A
XX, PNC	48D–49A; 59A–60E
$X\widehat{XY}$, PNC	58A–58F
3L chromosome	
XX, SG	68C–69D; 76B–3R; 71C–75C–77A; 70C–71C; 67A–67DE; 65DE–67A; 67DE–68C; 62F–64A; 65D–66A; 64C–67A; 70C–74A; 70C–71C
$X\widehat{XY}$, SG	67D–70C; 74A–75C; 64C–65E; 65D–67A; 65D–67D; 75C-chr; 79A–X; 64C–67D; 64C–67D; 67D–9A; 71C–74A; 70C–71C
XX, PNC	
$X\widehat{XY}$, PNC	75F–76B; 66E–67CD; 66E–67E; 69D–70A
3R chromosome	
XX, SG	86C–33B; 84F–36C; 88E–84A; 86D–85E; 81F–84A; 85A–86D; 92D–94A
$X\widehat{XY}$, SG	85B–87B; 83A–chr; 91D–93A; 83A–chr; 83D–87B; 89E–91C; 87D–92B; 81F–84E; 83A–chr
XX, PNC	98D–99A
$X\widehat{XY}$, PNC	

regions (see Figures 6–10). As to fine bands, further comparative electron microscopical studies are needed.

In general, the data obtained are in good agreement with previously stated views (Beerman 1952, 1972, Zhimulev & Belyaeva 1977, Zhimulev *et al.* 1983) regarding the relative constancy of banding pattern in normally functioning cells. The observed differences can be due to the stretching of interbands or splitting of bands (two or three bands in one tissue instead of one in another).

Acknowledgements

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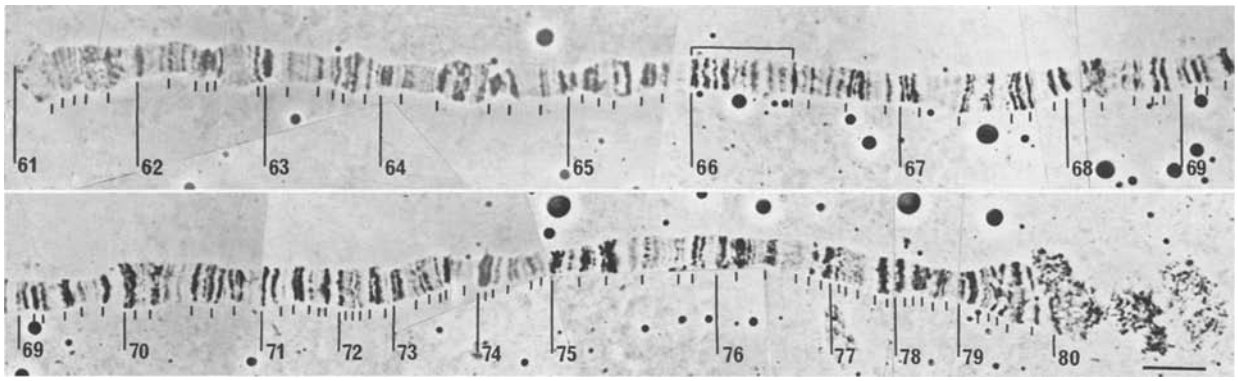

Figure 9. Photomap of the 3L chromosome from a PNC of an *otu*^{11} mutant. Brackets denote regions where no similarity with the SG polytene chromosomes was found. Bar = 10 μm.

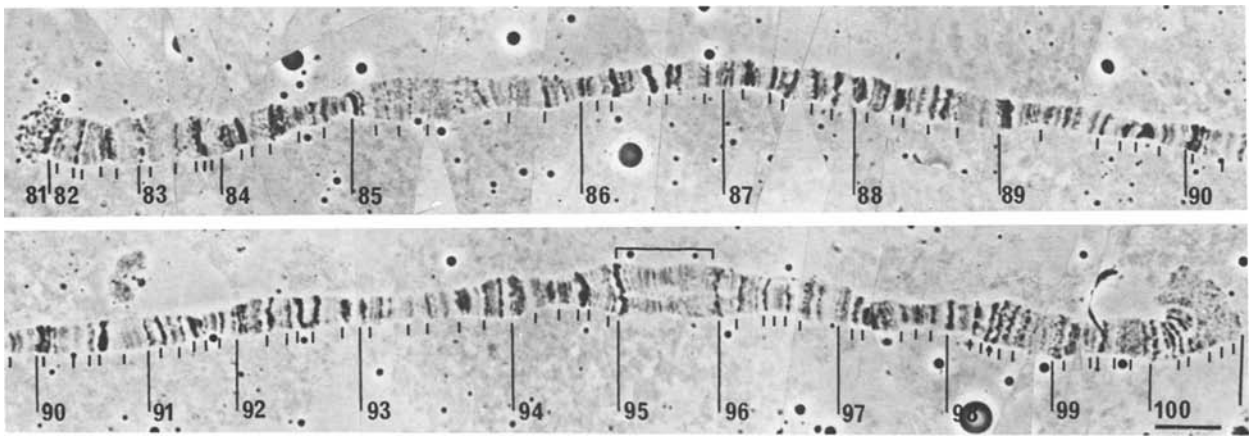

Figure 10. Photomap of the 3R chromosome from a PNC of an *otu*^{11} mutant. Brackets denote regions where no similarity with the SG polytene chromosomes was found. Bar = 10 μm.

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