# Electron microscopic band-interband pattern of polytene chromosomes in *Drosophila nasuta albomicans* 1 Salivary gland chromosome 2R

Om Prakash Singh* and Wolf-Ekkehard Kalisch

Institut für Genetik, Medizinische Fakultät, Ruhr-Universität Bochum, Postfach 10 21 48, D-4630 Bochum 1, Federal Republic of Germany
*Permanent address: Department of Zoology and Entomology, School of Agricultural Sciences and Rural Development, North-Eastern Hill University, Medziphema 797106 (Nagaland), India

## Abstract

The band-interband pattern of salivary gland chromosome 2R in *Drosophila nasuta albomicans* (division 53–83) was studied by light (LM) and electron microscopy (EM) using squash preparations and surface-spread polytene (SSP) chromosome preparations, respectively. LM and EM maps were compiled. Based on the digitized EM patterns of five homologous SSP chromosomes a computerized chromosome map was plotted. The EM pattern analysis showed a total number of 662 chromosome bands with an almost 98% increase compared with the LM analysis of squash preparations. The majority (about 92%) of interband lengths in SSP chromosome 2R ranged between 0.25 and 0.64 μm, which equal about 0.8–2.1 kb of totally extended DNA or 2.5–6.4 kb of DNA, if a DNA packing ratio of 0.1 μm/kb is assumed for the interbands of SSP chromosomes.

## Introduction

*Drosophila nasuta albomicans* is one of the twelve members of the *nasuta* subgroup of the *immigrans* species group (Wilson *et al.*, 1969; Ranganath and Hägele, 1981; Daniels *et al.*, 1990). During the last two decades, the *nasuta* subgroup has focused interest by several evolution, genetic, biochemical and molecular studies (*cf* Kalisch, 1991).

The subgroup members *D. n. albomicans* and *D. n. nasuta* are anisohomosequential (Ranganath and Krishnamurthy, 1974; Yoon, 1989): the mitotic chromosomes of both members show anisogenetic differences [*D. n. nasuta*, 2n = 8; *D. n. albomicans*, 2n = 6 by fusion of chromosome 3 with the gonosome. Further differences exist in the amounts of heterochromatin and in the size of chromosome 4 (Ranganath and Hägele, 1982)]. The band-interband patterns of the polytene chromosomes in both members are almost homosequential on the light microscropic (LM) level [the polytene chromosome complement of both members consists of five chromosomes designated X, 2L, 2R, 3 and 4. These comprise of a very long third chromosome, three medium sized chromosomes (X, 2L and 2R) and a very small fourth chromosome (Lin *et al.*, 1974; Ranganath and Krishnamurthy, 1974)]. However, there are no electron microscopic (EM) analyses of the band-interband patterns which prove that the homosequential patterns at the EM level exist.

In this paper, we revise the earlier LM chromomeric data of chromosome 2R (Sajjan and Krishnamurthy, 1973; Lin *et al.*, 1974; Ranganath and Krishnamurthy, 1974; Lambert, 1976; Roy and Lakhotia, 1981) by compiling a new LM map and counting the polytene structures in the individual sub-





Published and © 1991 by The Faculty Press
88 Regent Street, Cambridge, Great Britain

divisions. We also present an EM map based on SSP chromosome preparations and compare the patterns in both the maps. A computerized EM chromosome map has been plotted based on the digitized patterns of five homologous SSP chromosomes, and we use these data for an analysis of interband lengths. It is suggested that more than 92% of the interbands in *D. n. albomicans* are long enough to host the DNA-sequence of an average eukaryotic gene. These data coincide with earlier data on interband lengths in *D. hydei* (Kalisch *et al.*, 1986a,b).

Our study is to be seen as a first approach to a future EM comparison of the band-interband patterns between *D. n. albomicans* and *D. n. nasuta*, as well as a basis for any further cytological work in the *nasuta* subgroup.

## Materials and methods

The *Drosophila nasuta albomicans* wild-type strain used in this study was obtained from the National *Drosophila* Species Resource Center, Bowling Green, Ohio, U.S.A. (stock number 15112-1751). Larvae were raised on standard medium at 25 ± 1°C. Extra baker's yeast was provided to optimize the development up to the late third-instar.

### Squash preparation and light microscopic pattern analysis

The standard technique with alcohol and acetic acid (3:1) was used for squash preparations of salivary gland chromosomes. For the LM band-interband pattern analysis and the light micrographs of the unstained chromosomes in squash preparations, we used a Neofluar objective 100/1.3 Ph, Zeiss and a Neofluar 63/1.25 Ph, Zeiss, respectively.

### Surface-spread polytene (SSP) chromosome preparation

Salivary glands were excised and processed for SSP chromosome preparations according to the standard technique for *D. melanogaster* (Kalisch and Whitmore, 1986) after slight *nasuta*-specific modifications (Singh and Kalisch, 1991a): 3.74 M citric acid (12 g dissolved in 15 ml of *aqua bidist*) was used in the salivary gland pretreatment solution instead of the standard 3.18 M concentration. For the final composition of the pretreatment solution, 15 ml of citric acid (stock solution 3.74 M) was mixed with 30 ml of propionic acid (8.82 M, *i.e.* 19.8 ml propionic acid + 10.2 ml *aqua bidist*).

After 5—7 min of pretreatment, chromosomes were spread on the surface of a drop of the spreading medium (mixture of 4 M urea and 0.1 M HCl solutions) and picked up on a Formvar coated grid with a central 500 x 1,000 $\mu$m mesh. Grid coating with 0.35% Formvar solution provides a film strong enough to observe the preparations up to an EM magnification of x3,000.

### Electron microscopic pattern analysis

The SSP chromosomes were observed (without using any contrast medium) with a Siemens Elmiskop 101 at 60 kV and 80 kV and photographed on Elford technical film (0.18 mm polyester base) at x1,600 magnification. The final magnification of the micrographs used in the photo map was x2,800. The band-interband pattern was analysed from the original micrographs.

### Chromosome mapping

Sectioning of the LM and EM maps in Figure 1 is based on the LM map of *D. n. nasuta* (Ranganath and Krishnamurthy, 1974) which is seen to be almost homosequential with *D. n. albomicans* (Rajasekarasetty *et al.*, 1978, 1980). According to the mapping of *D. n. nasuta*, chromosome 2R of *D. n. albomicans* is divided into thirty-one divisions labelled from 53 to 83.

In the earlier *D. n. nasuta* LM map (Ranganath and Krishnamurthy, 1974) there was no sub-divisioning. We mapped the subdivisions according to the needs for EM computerized band-interband patterns (Kalisch *et al.*, 1984). Each band in the EM map of Figure 1 is denoted by a dot and the total number of bands counted in each sub-division is mentioned at the right hand margin. Total numbers of bands including closely juxtaposed and dotted bands are given in Table 1. Bands of similar thickness and band type, with interbands not greater than 0.094 $\mu$m (Kalisch *et al.*, 1986b), have been listed as doublets and triplets in Table 1.

### Digitization and computerized plotting of the chromosome map

In order to plot a computerized EM chromosome map, measurements of polytene structures [band thicknesses, band diameters, interband lengths, band types (dotted) according to the approximate DNA content of the chromomeres, and characteristic marker-bands] were taken from five different electron micrographs using a magnified measuring scale of 0.1 mm unit and pattern parameters defined earlier (Kalisch *et al.*, 1984). The computer and plotter hardware used [IBM PC XT (MS-DOS; BASIC); HP 7475A plotter (pen diameter, 0.3 mm; minimum pen shift width, 0.025 mm)] as well as the digitizing and plotting programs are the same as in earlier studies (Kalisch *et al.*, 1984, 1986a,b).

## Results

### LM and EM pattern analysis

Cytogenetic identification of chromosome 2R is favoured by the constantly found loop configuration (division 53) at the proximal end. This chromosome configuration is formed by multiple ectopic pairing among the bands of the subdivisions 53A, 53E, 54A and 55A (Singh and Kalisch, 1991b). The tip of chromosome 2R is characterized by a fan-shaped expansion of the terminal bandgroup. Both land-marks are to be found in squash preparations as well as in SSP chromosome preparations (Figure 1).

Division sectioning of the LM map in Figure 1 is based on an earlier LM map of the almost homosequential *D. n. nasuta* strain (Ranganath and Krishnamurthy, 1974). The subdivision sectioning presented in Figure 1 follows the parameters for computerized chromosome mapping (Kalisch *et al.*, 1984) concerning the position of the section borders in relation to the interbands (*cf* EM chromosome map in Figure 2).

The LM map in the four plates of Figure 1 shows one and the same chromosome, whereas the EM map in the same Figure is compiled from one SSP

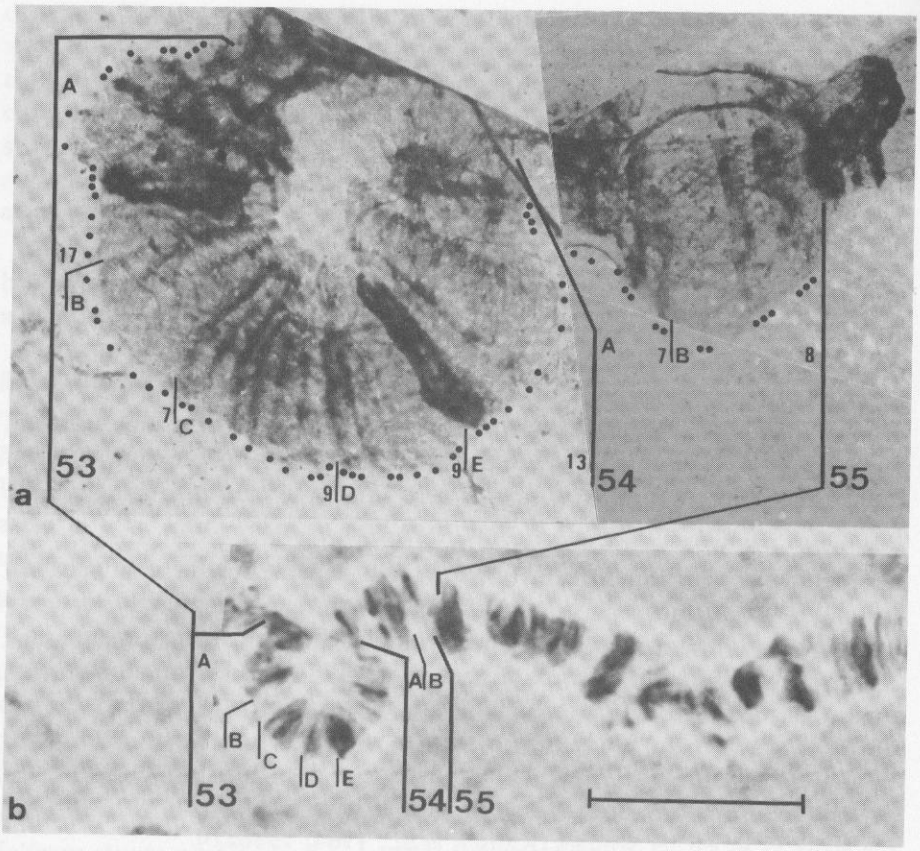


**Figure 1** EM (a) and LM (b) maps of chromosome 2R in *Drosophila nasuta albomicans.* Division 53—83, arranged on four sequential plates (53—54, 55—66, 67—76 and 77—83). The LM map comes from one squash preparation. In the EM map, subdivisions 53A—E, 61B—66C and 73B—76B have been replaced by three different SSP chromosome preparations. The constantly found loop configuration of subdivision 53 (Singh and Kalisch, 1991b) has been arranged in the EM map in a similar position to be seen in the LM map. Division sectioning is based on the LM reference map of the almost homosequential *D. n. nasuta* strain (Ranganath and Krishnamurthy, 1974). Each dot in the EM map labels one chromosome band identified in the original EM micrograph. The total number of bands are given at the right border in each subdivision. The bar scales equal 10 $\mu$m in both LM and EM maps.

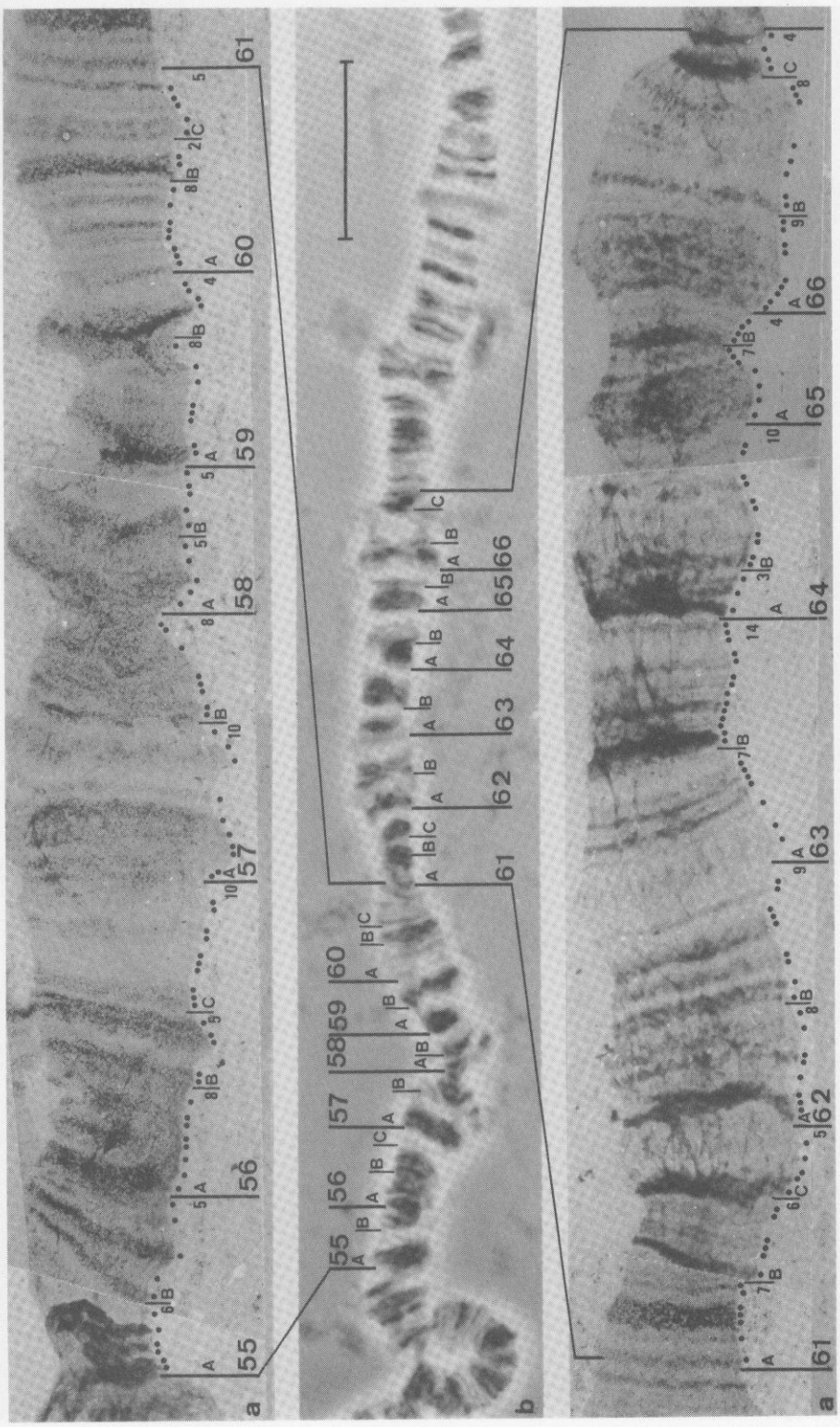


**Figure 1** Continued

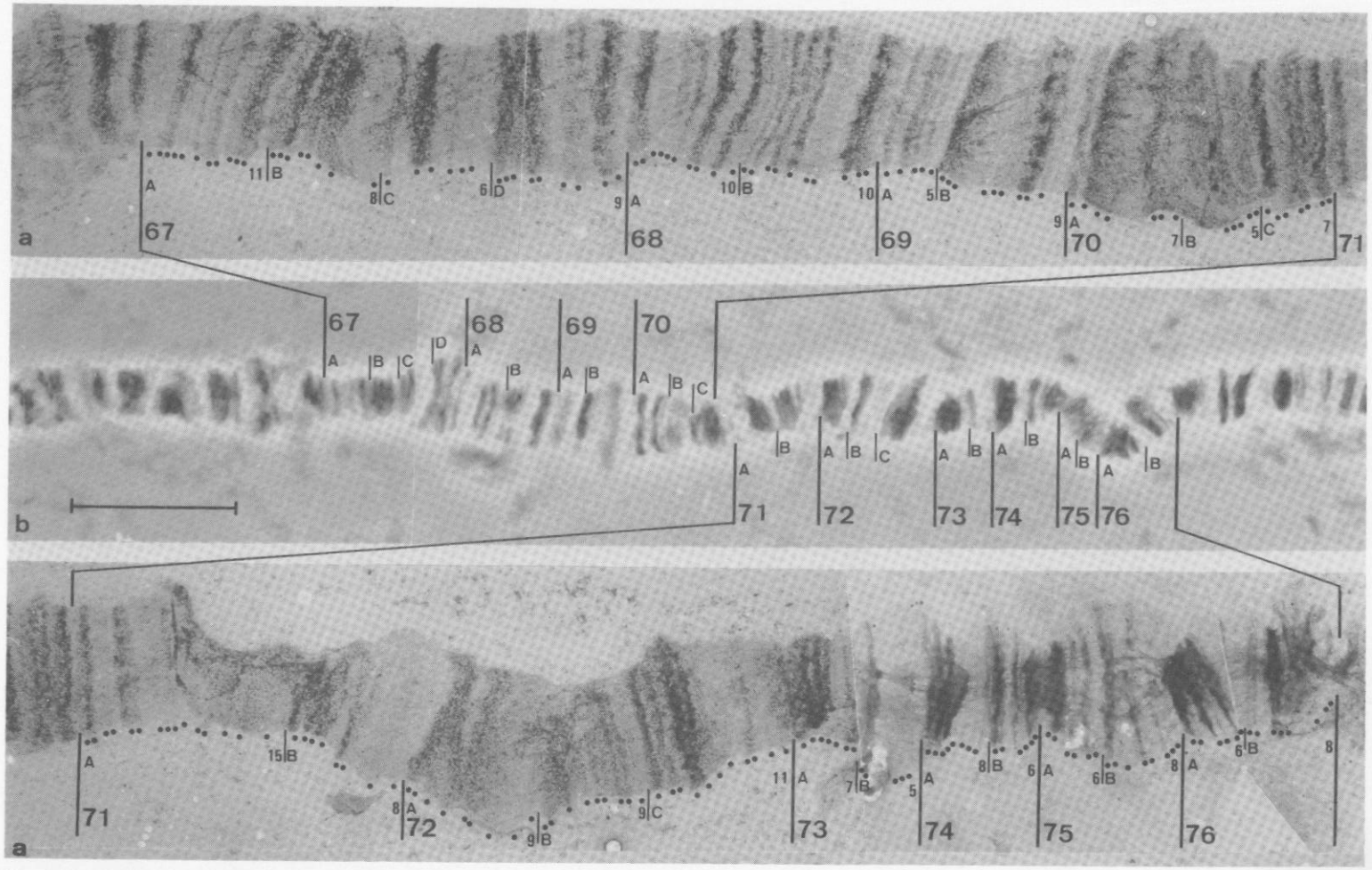


**Figure 1** Continued

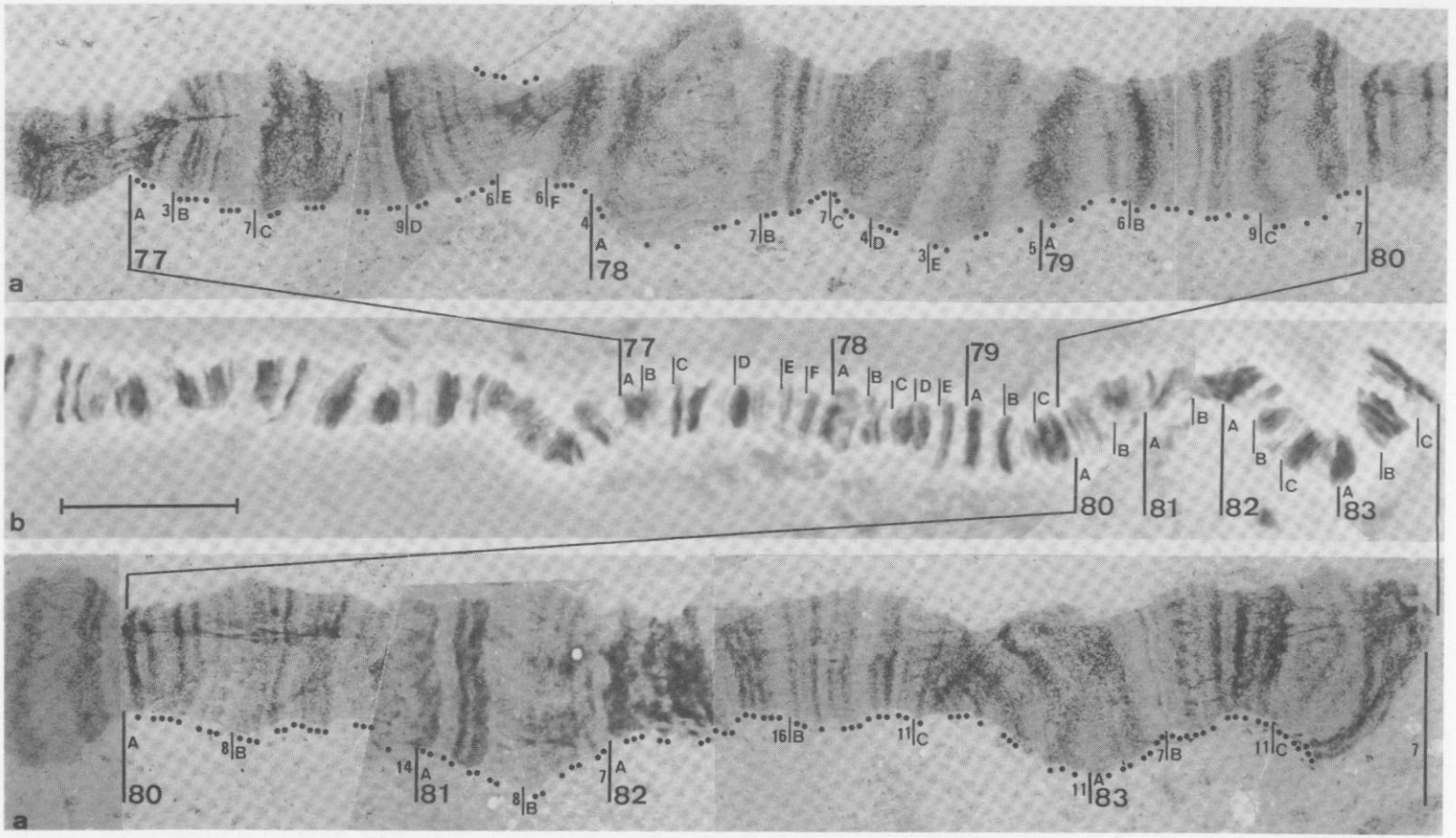


**Figure 1** Continued

chromosome preparation in which three regions are replaced by material from three different SSP chromosome preparations: subdivisions 53A—E, 61B—66C and 73B—76B. In the LM and EM maps depicted from third-instar developmental stages the puff patterns are somewhat different (*e.g.* puffs in 96B and 79C).

The average lengths and widths of chromosome 2R in squash preparations are about 165 and 3.4 $\mu$m, respectively. In SSP chromosome preparations with a comparable degree of polyteny, an average chromosome size of 500 $\mu$m in length and 10.7 $\mu$m in width is found. This indicates an almost three-fold longitudinal and lateral expansion of the giant chromosomes by the SSP chromosome preparation technique used. Comparable spreading degrees of salivary gland chromosomes using the same technique have also been found in several *Drosophila* and *Chironomus* species (Kalisch and Whitmore, 1983; Kalisch *et al.*, 1985). In Figure 1, the LM and EM maps are depicted at the same magnification which illustrates the differences in pattern resolution between common squash preparation and SSP chromosome preparation.

In the majority of the SSP chromosome preparations, chromosome subdivisions 53E—54A, 57B—58A, 66C, 71A—B and 76B—77A showed conspicuous constrictions. Constrictions are generally characterized by different cytogenetic peculiarities: ectopic pairing points, weak points, underreplicated chromosome sections, intercalary heterochromatin and/or late replicating polytene structures (Kalisch and Hägele, 1976). In chromosome subdivisions 53E—54A, 58A and 76B—77A the weak point character is obvious from stretched parts in squash preparations and excessively spread in SSP chromosome preparations where these regions were often found broken or weakly connected.

The number of bands (Table 1) were counted light microscopically in the original chromosome from which the LM map is depicted (Figure 1). The total number of 335 chromosome bands has to be compared with the 315 bands in an earlier chromosome map of the almost homosequential chromosome 2R in *D. n. nasuta* (Sajjan and Krishnamurthy, 1973). Unfortunately, any light micrograph of squash preparations shows only a reduced pattern compared with the direct LM analysis of the chromosome itself. The LM pattern resolution in earlier light micrographs of *D. n. nasuta* and *D. n. albomicans* in which the total number of bands is not listed (Lin *et al.*, 1974; Ranganath and Krishnamurthy, 1974; Roy and Lakhotia, 1981) cannot be compared with the analysis of our present study.

The EM map in Figure 1 indicates a total band number of 635 compared with 662 in the chromosome map of Figure 2 based on five homologous chromosome preparations analysed. This indicates that even in the EM analysis of individual SSP chromosome preparations of *D. n. albomicans* a certain amount of faint bands and interbands cannot be identified.

#### EM chromosome map

The EM band-interband pattern of chromosome 2R, based on the average data pooled from five SSP chromosome preparations, has been included in the

computerized chromosome map in Figure 2. In comparison with other computerized chromosome maps (Kalisch *et al.*, 1986a,b), the interband lengths of the chromosome map in Figure 2 do not represent the relationships to be seen in the original micrographs. Interband lengths are relatively shortened (in comparison with that found in the EM map of Figure 1), in order to reduce the total length of the chromosome.

To mimic the different DNA/protein contents of individual faint or diffused bands, these polytene structures are represented by dotted lines with different numbers of dots used in individual polytene structures of the chromosome map in Figure 2. In cases, where the pattern gets complex by closely juxtaposed dotted bands, the total number of bands can be checked in Table 1. The LM map used as reference map for the EM chromosome map in Figure 2 is identical with the one in Figure 1.

A subdivision-wise comparison of the band numbers between LM and EM analyses has been listed in Table 1. The number of bands in the EM analysis indicates about 98% increase compared with the LM analysis. This was achieved both by the technique of SSP chromosome preparation as well as by the higher EM resolution.

#### Quantitative analysis

Measurements of interband lengths in the electron micrographs of SSP chromosomes 2R indicated that about 92% interbands are in the range of 0.25 to 0.64 $\mu$m (the decision on range selection was made by the distribution of measurement values). Another 3% of interbands are longer than 0.64 $\mu$m (maximum 1.5 $\mu$m) and the remaining 5% are shorter than 0.25 $\mu$m. A range of 0.25 to 0.64 $\mu$m equals about 0.8—2.1 kb of totally extended DNA. If a DNA packing ratio of 0.1 $\mu$m/kb is assumed for SSP chromosomes (Kress *et al.*, 1985), then the interband lengths should represent about 2.5—6.4 kb of DNA. In both ways the DNA content of interbands coincide with the average DNA length of a eukaryotic gene.

## Discussion

For the first time, a complete band-interband pattern analysis of an entire polytene chromosome is given in one and the same paper, including the LM and the EM maps together with the EM chromosome map. The side-by-side arrangement of the LM and the EM maps in the same figure (Figures 1 and 2) facilitates a direct comparison between these two levels of pattern resolution.

Several LM analyses of the band-interband pattern in the almost homosequential salivary gland chromosomes of *D. n. nasuta* and *D. n. albomicans* have already been published as photo maps (Sajjan and Krishnamurthy, 1973; Lin *et al.*, 1974; Ranganath and Krishnamurthy, 1974; Lambert, 1976; Roy and Lakhotia, 1981). Unfortunately, different division sectioning used in each map makes them distinct from the other ones. It is extremely difficult to correlate cytogenetic data from different analyses. In order to overcome such discrepancies

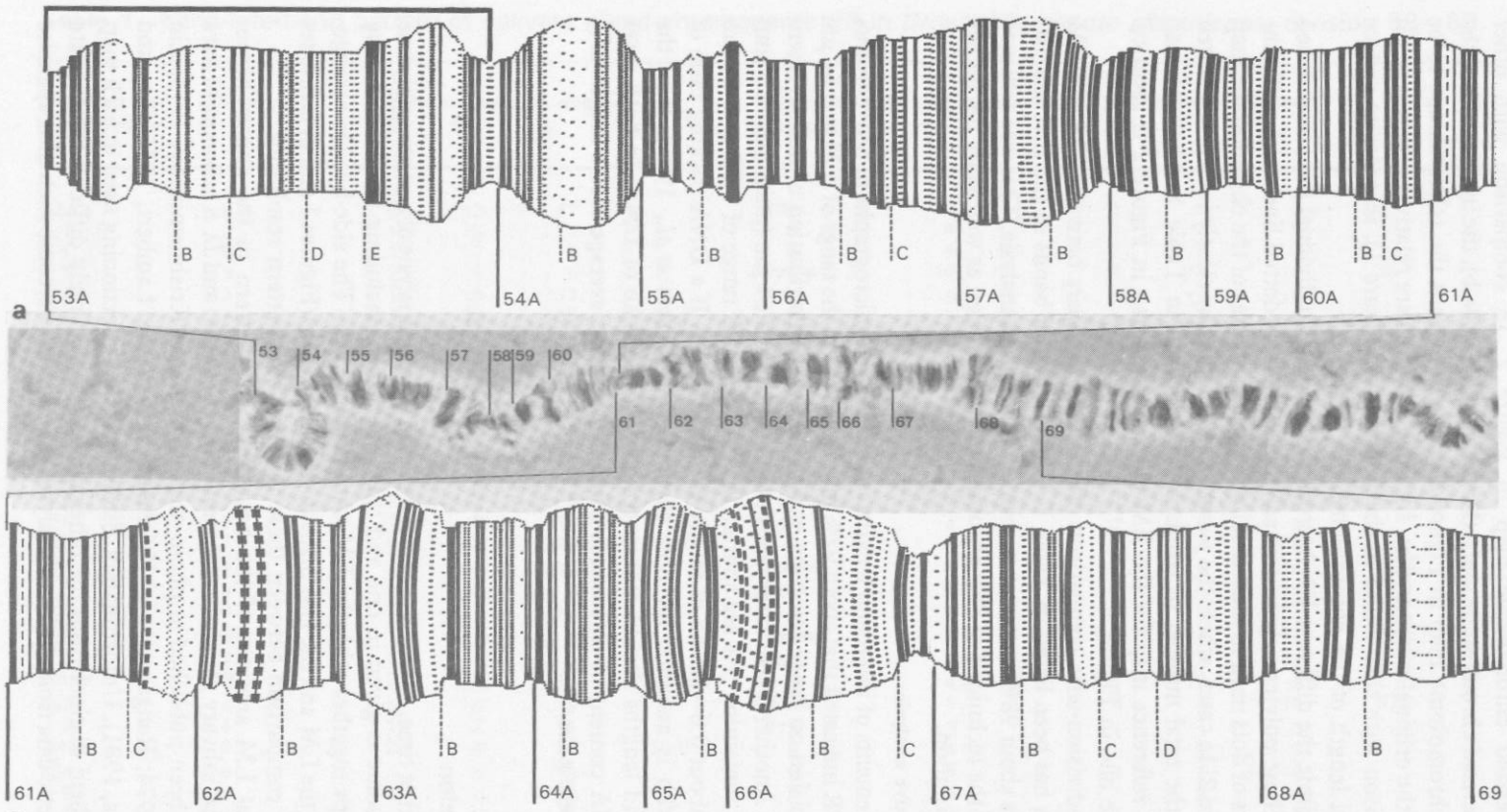


**Figure 2** EM chromosome map of salivary gland chromosome 2R in *Drosophila nasuta albomicans*: division 53—83. Band thickness and band diameter of individual polytene structures depicted in this map are average values based on measurements in electron micrographs of five SSP chromosomes. Interband lengths in relation to the average values in EM micrographs are reduced. The reference map is identical with the LM map in Figure 1. The bracket above division 53 indicates constantly found loop configuration (Singh and Kalisch, 1991b). Total numbers of bands including closely juxtaposed and dotted bands are given in Table 1.

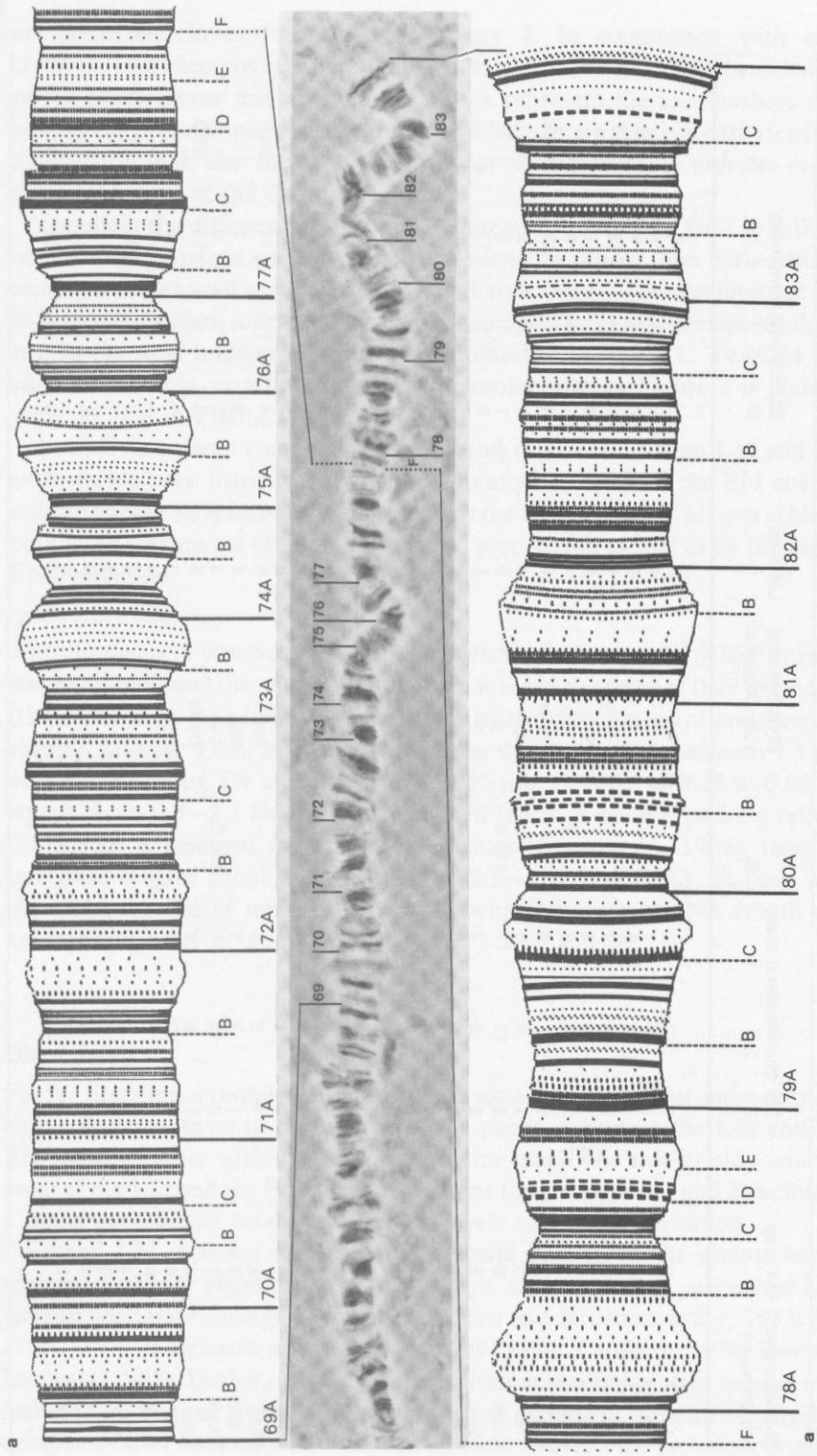


**Figure 2** Continued

**Table 1** Band-interband pattern of salivary gland chromosome 2R in *Drosophila nasuta albomicans:* division 53–83

| Divisions and sub-divisions | LM map t | EM chromosome map: D | S | t | % | Divisions and sub divisions | LM map t | EM chromosome map: D | S | t | % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 53A | 6 | 2 | 13 | 17 | | 70A | 6 | 1 | 5 | 7 | |
| B | 3 | 1 | 5 | 7 | | B | 4 | 1 | 3 | 5 | |
| C | 5 | 1 | 7 | 9 | | C | 4 | 2 | 3 | 7 | |
| D | 3 | 2 | 5 | 9 | | Totals | 14 | 4 | 11 | 19 | 35.7 |
| E | 12 | 1T + 1 | 8 | 13 | | 71A | 5 | 2 | 11 | 15 | |
| Totals | 29 | 1T + 7 | 38 | 55 | 89.7 | B | 7 | 1 | 6 | 8 | |
| 54A | 5 | 1 | 7 | 9 | | Totals | 12 | 3 | 17 | 23 | 91.7 |
| B | 3 | 1 | 6 | 8 | | 72A | 4 | 2 | 5 | 9 | |
| Totals | 8 | 2 | 13 | 17 | 112.5 | B | 5 | 1 | 7 | 9 | |
| 55A | 5 | 2 | 3 | 7 | | C | 5 | 2 | 7 | 11 | |
| B | 2 | — | 5 | 5 | | Totals | 14 | 5 | 19 | 29 | 107.1 |
| Totals | 7 | 2 | 8 | 12 | 71.4 | 73A | 4 | 1 | 5 | 7 | |
| 56A | 4 | 3 | 2 | 8 | | B | 3 | 1 | 3 | 5 | |
| B | 5 | 2 | 2 | 6 | | Totals | 7 | 2 | 8 | 12 | 71.4 |
| C | 2 | 1 | 8 | 10 | | 74A | 4 | 2 | 4 | 8 | |
| Totals | 11 | 6 | 12 | 24 | 118.2 | B | 5 | 1 | 4 | 6 | |
| 57A | 4 | 2 | 6 | 10 | | Totals | 9 | 3 | 8 | 14 | 55.6 |
| B | 5 | 1 | 6 | 8 | | 75A | 5 | 1 | 4 | 6 | |
| Totals | 9 | 3 | 12 | 18 | 100.0 | B | 3 | — | 8 | 8 | |
| 58A | 2 | 1 | 3 | 5 | | Totals | 8 | 1 | 12 | 14 | 75.0 |
| B | 3 | 1 | 3 | 5 | | 76A | 4 | 2 | 3 | 7 | |
| Totals | 5 | 2 | 6 | 10 | 100.0 | B | 4 | 1 | 6 | 8 | |
| 59A | 4 | 1 | 6 | 8 | | Totals | 8 | 3 | 9 | 15 | 87.5 |
| B | 4 | 1 | 2 | 4 | | 77A | 3 | 1 | 1 | 3 | |
| Totals | 8 | 2 | 8 | 12 | 50.0 | B | 4 | — | 7 | 7 | |
| 60A | 4 | — | 8 | 8 | | C | 4 | 2 | 6 | 10 | |
| B | 2 | 1 | — | 2 | | D | 5 | 1 | 4 | 6 | |
| C | 4 | — | 5 | 5 | | E | 3 | 1 | 4 | 6 | |
| Totals | 10 | 1 | 13 | 15 | 50.0 | F | 3 | — | 4 | 4 | |
| | | | | | | Totals | 22 | 5 | 26 | 36 | 63.6 |

**Table 1** (continued)

| Divisions and sub-divisions | LM map t | EM chromosome map: D | S | t | % |
|---|---|---|---|---|---|
| 61A | 5 | 1T + 1 | 3 | 8 | |
| B | 2 | 1 | 6 | 8 | |
| C | 4 | 1T + – | 4 | 7 | |
| Totals | 11 | 2T + 2 | 13 | 23 | 109.1 |
| 62A | 4 | 1 | 6 | 8 | |
| B | 4 | 2 | 7 | 11 | |
| Totals | 8 | 3 | 13 | 19 | 137.5 |
| 63A | 4 | 1 | 5 | 7 | |
| B | 5 | 2 | 10 | 14 | |
| Totals | 9 | 3 | 15 | 21 | 133.3 |
| 64A | 4 | 1 | 2 | 4 | |
| B | 4 | 2 | 6 | 10 | |
| Totals | 8 | 3 | 8 | 14 | 75.0 |
| 65A | 3 | 1 | 6 | 8 | |
| B | 2 | 1 | 3 | 5 | |
| Totals | 5 | 2 | 9 | 13 | 160.0 |
| 66A | 2 | 2 | 6 | 10 | |
| B | 3 | 2 | 4 | 8 | |
| C | 3 | 1 | 2 | 4 | |
| Totals | 8 | 5 | 12 | 22 | 175.0 |
| 67A | 4 | 2 | 7 | 11 | |
| B | 3 | 2 | 5 | 9 | |
| C | 3 | 1 | 5 | 7 | |
| D | 4 | 2 | 6 | 10 | |
| Totals | 14 | 7 | 23 | 37 | 164.3 |
| 68A | 6 | 2 | 7 | 11 | |
| B | 5 | 1 | 9 | 11 | |
| Totals | 11 | 3 | 16 | 22 | 100.0 |
| 69A | 3 | – | 6 | 6 | |
| B | 4 | 2 | 6 | 10 | |
| Totals | 7 | 2 | 12 | 16 | 128.6 |
| 78A | 3 | 1 | 6 | 8 | |
| B | 3 | 1 | 5 | 7 | |
| C | 3 | – | 4 | 4 | |
| D | 2 | – | 3 | 3 | |
| E | 2 | 1 | 3 | 5 | |
| Totals | 13 | 3 | 21 | 27 | 107.7 |
| 79A | 3 | 1 | 4 | 6 | |
| B | 5 | 1 | 7 | 9 | |
| C | 3 | 1 | 5 | 7 | |
| Totals | 11 | 3 | 16 | 22 | 100.0 |
| 80A | 7 | 1 | 6 | 8 | |
| B | 5 | 2 | 10 | 14 | |
| Totals | 12 | 3 | 16 | 22 | 83.3 |
| 81A | 5 | 2 | 4 | 8 | |
| B | 4 | 1 | 5 | 7 | |
| Totals | 9 | 3 | 9 | 15 | 66.7 |
| 82A | 5 | 1T + 2 | 9 | 16 | |
| B | 5 | – | 11 | 11 | |
| C | 7 | 1 | 9 | 11 | |
| Totals | 17 | 1T + 3 | 29 | 38 | 123.5 |
| 83A | 3 | 1 | 5 | 7 | |
| B | 4 | 1 | 9 | 11 | |
| C | 4 | – | 8 | 8 | |
| Totals | 11 | 2 | 22 | 26 | 136.4 |
| Totals 53–83 | 335 | 4T + 98 | 454 | 662 | 97.6 |

Comparison between the LM analysis of the original chromosome preparation depicted in Figure 1 and the data of the EM chromosome map depicted in Figure 2, which are based on the EM analysis of five SSP chromosome preparations. t, Total number of bands; T, number of triplet bands; D, number of doublets; S, number of single bands; %, percentage increase of additional bands in the EM chromosome map.

and to provide generalized EM and LM reference maps, we compiled a new EM map and revised the LM maps. We used the sectioning modus of *D. n. nasuta* (Ranganath and Krishnamurthy, 1974), because the sectioning of thirty-one divisions used for chromosome 2R in this map allows us to divide each division into a small number of subdivisions which do not cover too many polytene structures.

EM band-interband pattern resolution in Figure 1 is limited to the somewhat indistinct characters of the chromosome bands. Modifications of the SSP chromosome preparation technique used during the last decade indicate that pattern resolution in SSP chromosome preparations depends not only on the degree of polyteny and the skilful handling of the preparation technique but also on the species-specific chromosomal proteins (Kalisch and Hägele, 1981).

As a result, the data analysed of the five homologous SSP chromosomes presented in this paper (Table 1 and Figure 2) cannot be considered final and should not be compared with larger studies (Kalisch *et al.*, 1985, 1986a,b) on *D. hydei* (an organism which is much more appropriate to the SSP chromosome preparation technique used). The present study should also not be compared with the very detailed studies on *D. melanogaster*, which lasted for about two decades probably due to the difficult technique of chromosome preparation used (*cf* Sorsa *et al.*, 1984; Saura *et al.*, 1988). For a comparable analysis in *Drosophila nasuta albomicans*, a collection of further data from different developmental stages are needed. However, we know from additional unpublished data of individual subdivisions of chromosome 2R that the increase of additional bands will be very low compared with the differences found between the LM and EM maps in the present study.

Our comparison of the band-interband patterns between the LM and the EM maps indicated an increased number of faint chromosome bands (due to the EM resolution) as well as an increased number of closely juxtaposed bands and interbands visible within prominent band groups (due to the SSP chromosome preparation technique). An increase of about 98% bands was recorded in the EM chromosome map compared with the LM analysis in the chromosomes of squash preparations. This indicates that computerized EM chromosome mapping is to be considered as a prerequisite for any detailed EM cytogenetic analysis, where data need comparison with a generalized band-interband pattern.

During the present study in *D. n. albomicans* we found interband lengths in the range of 0.24—0.62 $\mu$m. Regardless of the actual configuration of DNA in the interbands of SSP chromosomes (totally extended DNA or a DNA packing ratio of 0.1 $\mu$m/kb), the DNA content of more than 92% interbands coincides with the DNA value (1.0—1.7 kb) estimated for an average eukaryotic gene (Hovemann *et al.*, 1981; Benyajati *et al.*, 1983). Therefore, we assume, in contrast to other investigations (Sorsa, 1982), that the average interband in *D. n. albomicans* should be long enough for coding a eukaryotic gene. This is in accordance with the results of the earlier studies in *D. hydei* (Kalisch *et al.*, 1986a,b), where an average interband was measured at about 0.33 $\mu$m, *i.e.* 50% longer than the calculation based on squash preparations in *D. melanogaster* by Sorsa *et al.* (1984).

We also excluded the argument that band material is pulled into the interbands during the SSP chromosome preparation by pattern analyses in chromosomes which showed different degrees of longitudinal spreading (Kalisch and Whitmore, 1983; Kalisch *et al.*, 1986a,b).


## Acknowledgements

The authors wish to thank Mrs C. Plehn and Mrs S. Becker for technical assistance. O.P.S. is grateful to the North-Eastern Hill University for sponsorship and to the DAAD, Federal Republic of Germany, for the award of a fellowship.


## References


BENYAJATI C., Spoerel N., Haymerle H. and Ashburner M. 1983. The messenger RNA for alcohol dehydrogenase in *Drosophila melanogaster* in its 5′ end in different developmental stages. *Cell* **33** 125–33.

DANIELS S. B., Peterson K. R., Strausbaugh L. D., Kidwell M. G. and Chovnick A. 1990. Evidence for horizontal transmission of the P transposable elements between *Drosophila* species. *Genetics* **124** 339–55.

HOVEMANN B., Galler R., Walldorf U., Küpper H. and Bautz E. K. F. 1981. Vitellogenin in *Drosophila melanogaster*: sequence of yolk proteins and its flanking regions. *Nucleic Acids Res.* **9** 4721–34.

KALISCH W-E. 1991. Bibliography on the *D. nasuta* subgroup. *Drosophila Inf. Serv.* **69** In press.

KALISCH W-E. and Hägele K. 1976. Correspondence of banding patterns to 3H-thymidine labelling patterns in polytene chromosomes. *Chromosoma* **57** 19–23.

KALISCH W-E. and Hägele K. 1981. Surface spreading of polytene chromosomes. *Eur. J. Cell Biol.* **23** 317–20.

KALISCH W-E. and Whitmore T. 1983. Differences in the number of polytene chromosome bands as studied by electron microscopy. *Cytobios* **37** 37–43.

KALISCH W-E. and Whitmore T. 1986. The SSP chromosome preparation technique as applied for *D. melanogaster*. *Drosophila Inf. Serv.* **62** 142–6.

KALISCH W-E., Schwitalla G. and Whitmore T. 1986a. Electron microscopic band-interband pattern of the X chromosome in *Drosophila hydei*. *Chromosoma* **93** 387–92.

KALISCH W-E., Schwitalla G. and Whitmore T. 1986b. Computerized EM maps of surface-spread polytene chromosomes. 2. Chromosome 4 in *Drosophila hydei*. *Cytobios* **45** 185–94.

KALISCH W-E., Whitmore T. and Reiling H. 1984. Computerized EM maps of surface spread polytene chromosomes. 1. Digitizing and plotting of banding patterns. *Cytobios* **41** 47–62.

KALISCH W-E., Whitmore T. and Schwitalla G. 1985. Electron microscopic map of surface-spread polytene chromosomes in *Drosophila hydei*. *Chromosoma* **92** 265–72.

KRESS H., Meyerowitz E. M. and Davidson N. 1985. High resolution of *in situ* hybridized biotinylated DNA to surface-spread *Drosophila* polytene chromosomes. *Chromosoma* **93** 113–22.

LAMBERT D. M. 1976. The chromosomes of four species of the *nasuta* complex of *Drosophila*. I. Chromosome maps and inversion polymorphism. *J. Heredity* **67** 92–8.

LIN F-J., Tseng H-C. and Wang T-C. 1974. Standard map of the salivary gland chromosomes of *Drosophila albomicans* Duda. *Drosophila Inf. Serv.* **51** 42–3.

RAJASEKARASETTY M. R., Krishnamurthy N. B. and Ranganath H. A. 1978. Population genetics of *D. n. nasuta* and *D. n. albomicans* and their hybrids. II. Coassociation of parental chromosomes in the hybrid populations. *Proc. Ind. Acad. Sci.* **87** 249–55.

RAJASEKARASETTY M. R., Ramesh S. R. and Krishnamurthy N. B. 1980. Interspecific chromosomal variation among a few members of the *nasuta* subgroup (Genus: *Drosophila*). *Entomon* **5** 1–12.

RANGANATH H. A. and Hägele K. 1981. Karyotypic orthoselection in *Drosophila*. *Experientia* **68** 527–8.

RANGANATH H. A. and Hägele K. 1982. The chromosomes of two *Drosophila* races: *D. nasuta nasuta* and *D. n. albomicana*. I. Distribution and differentiation of heterochromatin. *Chromosoma* **85** 83–92.

RANGANATH H. A. and Krishnamurthy N. B. 1974. Chromosomal morphism in *Drosophila nasuta* Lamb. Photomap of salivary gland chromosomes and microdifferentiation in the polymorphism of Mysore populations. *J. Mysore Univ.* **26B** 65–9.

ROY S. and Lakhotia S. C. 1981. Replication in *Drosophila* chromosomes. IV. Patterns of chromosomal replication in salivary gland polytene nuclei of *Drosophila nasuta. Ind. J. Expt. Biol.* **19** 797–807.

SAJJAN S. N. and Krishnamurthy N. B. 1973. Salivary gland chromosome map of *Drosophila nasuta* (Lamb). *J. Mysore Univ.* **25B** 28–31.

SAURA A. O., Heino T. I. and Sorsa V. 1988. Electron microscopic map of the division 74 through 80 of the salivary gland 3L chromosome in *Drosophila melanogaster. Hereditas* **109** 1–13.

SINGH O. P. and Kalisch W-E. 1991a. SSP technique applied for EM genome analysis and photo mapping in *Drosophila nasuta. Drosophila Inf. Serv.* **69.** In press.

SINGH O. P. and Kalisch W-E. 1991b. Ectopic pairing responsible for a constantly found 'loop' configuration in polytene chromosomes of *Drosophila. Caryologia.* In press.

SORSA V. 1982. Electron microscopic map for the salivary gland chromosome X of *Drosophila melanogaster.* Division 1–5. *In* Advances in Genetics, Development and Evolution in *Drosophila.* pp 23–31. Edited by S. Lakovaara. Plenum Publishing Corporation, New York.

SORSA V., Saura A. O. and Heino T. I. 1984. Electron microscopic map of the division 61, 62 and 63 of the salivary gland 3L chromosome in *Drosophila melanogaster. Chromosoma* **90** 177–84.

WILSON F. D., Wheeler M. R., Harget M. and Kambysellis M. 1969. Cytogenetic relations in the *Drosophila nasuta* subgroup of *immigrans* group of species. *Univ. Texas Publ.* **6918** 207–53.

YOON J. S. 1989. Chromosomal evolution and speciation in Hawaiian *Drosophila. In* Genetics, Speciation and the Founder Principle. pp 129–47. Edited by L. V. Giddings, K. Y. Kaneshiro and W. W. Anderson. Oxford Univ. Press.

Accepted 12 October 1990

## News to authors

We can process your manuscripts for CYTOBIOS and MICROBIOS rapidly providing the typescript and illustrations have been carefully and accurately prepared in the correct style.

Papers for *MICROBIOS LETTERS* will be published very swiftly, and in many instances take only about eight to ten weeks from acceptance to publication. In general, manuscripts for *MICROBIOS LETTERS* should not exceed 5,000 words in length, and include only one or two Tables and/or Figures. Those papers which are too long for *MICROBIOS LETTERS* on submission will be considered for either MICROBIOS or CYTOBIOS.

By following the style of our biomedical journals meticulously you can obtain the advantages of some of the most rapid publication rates for research papers available anywhere.

Read the leaflets prepared for authors, entitled *Information for contributors* and *Photographic illustrations,* and send manuscripts for the biomedical journals MICROBIOS, CYTOBIOS and *MICROBIOS LETTERS* to *The Executive Editor, The Faculty Press, 88 Regent Street, Cambridge, England.*

* MICROBIOS, CYTOBIOS and MICROBIOS LETTERS are all prestige biomedical research journals.

* Fifty reprints are provided free.

* World-wide distribution, so authors invariably receive many requests for reprints.

* Three volumes of each title are published annually. Each volume consists of three to four issues.

* Subscription rates and leaflets for authors are available from *The Faculty Press, 88 Regent Street, Cambridge, England.*

**THE FACULTY PRESS 88 Regent Street Cambridge England**

## MICROBIOS

is an international biomedical research journal devoted to fundamental studies of viruses, bacteria, micro-fungi, microscopic algae, and protozoa. It is concerned with all aspects of micro-organisms, but lays particular emphasis upon chemical microbiology.

Original observations are accepted on the applications of microbiology in the fields of pharmaceutical and chemical production; food manufacture and spoilage; public health and sanitation; biodeterioration; pharmacology and immunology.

Papers on the organisation and metabolic activities of micro-organisms are published, as well as work on cell-virus interactions. Manuscripts which are especially welcome are those dealing with the chemical anatomy of micro-organisms, and the biochemical and biophysical factors that affect microbial activity.

## CYTOBIOS

is a trans-world biomedical research journal which publishes original investigations into all aspects of cell organization. Contributions will be accepted on the behaviour, structure and function of animal and plant cells, including studies on extra-cellular products and subcellular organelles.

The journal emphasizes work at chemical and molecular levels. It publishes original papers on cytogenetics; cell division and growth; cell physiology and pathology; immunochemistry and immunobiology. Manuscripts are solicited which correlate findings in the biochemical and biophysical fields with morphological, cytological and physiological knowledge.

Discoveries resulting from advances in, and application of, modern biological and medical techniques to cytology are particularly welcome. So also are cytochemical papers which contribute to an understanding of cell organization and to the study of organic fine structure.

## MICROBIOS LETTERS

is a biomedical research journal having the fundamental aim of very rapid publication, and distribution to a wide international readership. It is intended for short and preliminary communications, but may include some longer papers and reviews. In general manuscripts should not exceed 5,000 words in length and include only one or two Tables and/or Figures.

Although primarily designed to augment MICROBIOS covering any microbiological studies, MICROBIOS LETTERS will consider papers in such fields as cellular pharmacology, virology, bacteriology, biochemistry, immunology, molecular biology, biochemical genetics, and biophysics.

**THE FACULTY PRESS 88 Regent Street Cambridge England**